Diet and heavy metal uptake by two top predator species in the Tees Estuary

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ABSTRACT

The key aim of the thesis was to estimate metal uptake and its seasonal variation from the diet by two predators from the Tees Estuary, harbour seals, *Phoca vitulina* and cormorants, *Phalacrocorax carbo*. The reproductive success of the colony of harbour seals, that has been re-establishing in the Tees Estuary since the late 1980s, has been poor and metal loads may be a potentially limiting factor. The diet of the predators was assessed and metal concentrations within the prey species were analysed.

Median metal concentrations in Crustacea and fish species from the Tees Estuary were higher than reported in pristine estuaries. Maximum metal concentrations in some individuals suggested that hot spots still exist. There were differences in metal concentrations between species and season so the seasonal diet of the predator was important in determining metal intake rates. Metal concentrations tended to be highest in Crustacea, followed by pleuronectids and lowest in the gadids.

The seals and cormorants were opportunist foragers and their diet reflected the seasonal availability of gadids. They appeared to switch to alternative prey when gadid numbers in the Tees Estuary declined. Metal burdens in the diet of these predators were expected to be lower in the winter because gadids were the dominant prey. Individual predators had different dietary preferences and hence, metal body burdens in predators would be expected to vary accordingly.

Retention of metals in the Tees seals was estimated from daily metal burdens in the diet and the metal burden in the faeces. The estimated retention of metals was considered unlikely to cause an adverse effect on the seals. Further work is required however, to determine whether they bioaccumulate. Mercury concentrations were high in some body organs of two seal carcasses recovered from the Tees Estuary, although levels in predominant prey species were relatively low. Seal carcasses should be analysed where possible to measure metal concentrations, particularly mercury and organochlorine concentrations.

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I am grateful for the support and advice from my supervisor Dr Martyn Lucas. I would like to acknowledge the advice provided by my original first supervisor Professor Peter Evans, sadly deceased in September 2001. Thanks also to Dr Robert Baxter, who became my second supervisor. The technicians were extremely helpful, particularly Michael Bone and Eric Henderson from the Biology Department and Frank Davis from the Geography Department. Robin Wards' knowledge of cormorants in the field was helpful. I would like to acknowledge Graham Pierce of Aberdeen University for sharing his knowledge on fish skeletal parts and letting me study his collection of fish bones and otoliths. Thanks for the advice on metal concentrations in Crustacea from Phillip Rainbow which prompted me to write Chapter 5. Thanks also to Tom Mercer for helping with invertebrate identification.

I faced a number of personal traumas and challenges during the course of this PhD and would not have managed it if it were not for the tremendous support that I received from my partner Paul, my family and friends.

I would like to dedicate this PhD to my Dad who died before it was complete. It was his influence and that of my Mums' that has allowed me to progress so far and I am indebted to them. My son Oliver was born two weeks after I handed in. I hope I have made him proud.

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Declaration

The author declares that this PhD is her own work, unless acknowledged otherwise. The material in the thesis has not previously been submitted for a degree in this or any other University.

Glossary

Abbreviations

Jan	January	Feb	February
Mar	March	Apr	April
May	May	Jun	June
Jul	July	Aug	August
Sept	September	Oct	October
Nov	November	Dec	December

As	Arsenic	Cd	Cadmium
Cr	Chromium	Cu	Copper
Hg	Mercury	Mn	Manganese
Ni	Nickel	Pb	Lead
Zn	Zinc		

C. Shr	Common Shrimp	Sh.Crab	Shore Crab
Sw. Crab	Swimming Crab	Wh	Whiting
Sai	Saithe	Fl	Flounder
Pl	Plaice	Herr	Herring
Sp	Sprat	Weev	Weever
SE	Sandeel		

E	Exoskeleton	HP	Hepatopancreas
M	Muscle	OP	Other parts

FAAS	Flame Atomic Absorption Spectrometry
MT	Metallothionein

Statistical terms

NS	=	No significant difference ($p \geq 0.05$)
*	=	Significant difference ($p \leq 0.05$)
**	=	Highly significant difference ($p \leq 0.01$)
***	=	Very highly significant difference ($p \leq 0.001$)
n	=	Number of samples

Structure of the PhD thesis

Chapter 1 includes the general introduction, the ecology of the biota of the Tees Estuary and the aims and the objectives. The general introduction provides a description of the history of the Tees Estuary and an account of the historical trends of pollution in the Tees Estuary. The ecology of the biota in the Tees estuary details the population numbers, diversity and annual changes in benthic fauna, crustacea, fish, harbour seals, *Phoca vitulina* and cormorants, *Phalacrocorax carbo* of the Tees Estuary. The general ecology of Crustacea, fish, cormorants and seals are also discussed. The monitoring data from the Tees Seals Research Programme, 1989-2003 is provided and summarised. The aims and objectives provide the purpose of this study.

Chapters 2 and 3 evaluate the prey preference of two key top predators of the Tees Estuary, seals and cormorants. Chapter 2 examines the development and use of a reference collection of fish and crustacean skeletal remains to identify prey species ingested and to estimate the frequency and size of prey consumption. The advantages and limitations of methods to determine diet are discussed. In Chapter 3 the main prey species in the diet of seals and cormorants from the Tees Estuary are identified and compared. The number and body sizes of each species consumed seasonally are quantified.

Chapters 4 and 5 explore the heavy metal concentrations in fish and crustaceans from the Tees Estuary. Chapter 4 provides a review on the effects of heavy metals on estuarine biota and discusses the effects of zinc, copper, lead, cadmium, arsenic and chromium concentrations on fish and Crustacea species and mercury concentrations in whiting and flounder from the Tees Estuary. The influences of several factors were assessed, including within and between species variation and the effect of body size, season and annual differences in metal concentrations. Metal concentrations in Crustacea and fish in the present study and previous studies in the Tees Estuary are compared. Chapter 5 determines the distribution of heavy metals in crustacean and fish tissues and discusses the implications of this for dietary uptake by top predators.

Chapters 6 and 7 review the effects of heavy metal concentrations in cormorants and seals, respectively. The heavy metal concentrations in seal body tissues and seal faecal

samples from the Tees Estuary are assessed. Seasonal metal burdens in top predators are estimated from daily metal burdens in the prey and metals excreted in seal faecal samples. These were compared with estimated metal burdens in the body tissues of two seals found dead on Seal Sands.

Chapter 8 summarises this study, provides a discussion concluding the results and proposes opportunities for further research.

CHAPTER 1. GENERAL INTRODUCTION

Estuaries are partially enclosed bodies of water formed where freshwater rivers flow into the ocean and mix with seawater (McLusky and Elliott, 2004). Fine sedimentary material is carried into the estuary from the sea and rivers and accumulates to form mudflats. Estuaries also receive high nutrient levels, stored in the water column and the sediment. The dynamic mixing of freshwater and saltwater is challenging to the physiology of animals and plants and only a few unique species are able to adapt to these stressful conditions (McLusky and Elliott, 2004). The low diversity of species reduces interspecific competition for the rich supply of nutrients available and hence, there is an abundance of tolerant species.

The abundance of euryhaline invertebrates supports large numbers of wading birds (McLusky and Elliott, 2004). Many estuaries in Britain have been designated to protect these winter feeding grounds. Protection is necessary since many of these unique and highly productive habitats are also used by man. Historically, ports were built on the banks of the estuary and industry, towns and cities were built up around them. Estuaries are also used for depositing effluent from industrial processes and domestic waste

The Tees Estuary is located on the north-east coast of England. The inter-tidal mud-flats of the Tees Estuary are the only ones remaining in the northeast coast between the Humber 140 km to the south and Fenham Flats 130 km to the north. The mudflats on the north bank of the Tees Estuary, known as Seal Sands (NZ 522 255) was notified in 1966 under Section 23 of the National Parks and Access to the Countryside Act (1949) as a Site of Special Scientific Interest (SSSI) and was re-notified in 1983/4 under section 28 of the Wildlife and Countryside Act (1981) (J.K. Smith, INCA, pers. comm). In 1995, Seal Sands SSSI was classified as part of the Teesmouth and Cleveland Coast Special Protection Area (SPA) under EC directive 79/409 on the Conservation of Wild Birds. Also in 1995, Seal Sands was designated as part of the Teesmouth and Cleveland Coast Ramsar Site Durham and Cleveland under the Ramsar Convention on Wetlands of International Importance especially as Waterfowl Habitat. All the above designations are as a consequence of the international and national importance of Seal Sands as a feeding and migration site for

wildfowl and waders. A detailed geography, history and natural history of the Tees Estuary will follow.

1.1 GEOGRAPHY OF THE TEES ESTUARY

The Tees Estuary receives freshwater from the River Tees and the River Leven. It passes through the industrial towns of Middlesbrough, Stockton and Billingham before flowing into the North Sea between the North and South Gare Breakwaters. Intensive industrialization commenced on the banks of the Tees Estuary during the early nineteenth century and it has been subject to many man-made changes since (Riddle and Lewis, 1999). Major sections have been straightened, tide training walls have been constructed and much of the lower estuary has been reclaimed. This has confined the estuary to a narrow channel which is regularly dredged to allow the entrance of ships.

At the mouth of the Tees estuary are inter-tidal mudflats, although less than 6% of the original inter-tidal mudflats of approximately 2400 hectares remain (Appendix A) (Parham, 1996). Land reclamation, to provide sites for industrial development, began soon after the construction of the North Gare breakwater in the 1890s and continued until 1974 (the last phase of the Tees Estuary reclamation process). These inter-tidal mudflats are the only ones remaining on the northeast coast between the Humber, 140 km to the south and Fenham Flats, 130 km to the north. Mudflats are found on both banks of the Tees Estuary; those to the south are known as Bran Sands and those to the north, Seal Sands (NZ 529260) (Appendix B). The Tees mudflats are habitat for a diversity of estuarine wildlife and are designated due to their importance as feeding and migration sites for wildfowl and waders.

The Tees Barrage was constructed across the estuary, approximately 2 km downstream of Stockton. Construction began on the 4th November 1991 and was completed on the 22nd April 1995. The barrage is built to a height above peak high tides, thus excluding the tide from the upstream section and reducing the tidal estuary from the former 44 km to only 18 km (Riddle and Lewis, 1999). The barrage acts as a weir, preventing the incursion of saline water upstream and forming a non-tidal freshwater river above the barrage. Flows, salinity

distribution and mixing characteristics of the estuary have been significantly affected by the construction of the barrage (Riddle and Lewis, 1999). The freshwater inflow to the estuary is now not necessarily continuous and tidal flows have been significantly reduced for a distance of approximately 9 km downstream of the barrage. Full salt water (34 Practical Salinity Units) now penetrates the estuary in the lower layer up to the barrage, whereas previous to the barrage the maximum salinity was 30. The barrage has resulted in strong layering of salinity between the waters in the estuary that originate from the river and the sea, forming an interface at a depth of approximately 1.5 m over a 11.5 km surveyed location. The major part of the vertical mixing now occurs at the mid-flood tide instead of the early part of the ebb tide. Fish that are able to withstand the immediate salinity change can move up and down stream using the fish pass.

1.2 HISTORICAL TRENDS OF POLLUTION IN THE TEES ESTUARY

In common with other rivers in populated parts of Britain, the River Tees was used as a depository for industrial and domestic effluents (Warwick *et al*, 2002). In 1970, the chemical industry on Teesside was thriving and the Tees estuary was classed as grossly polluted (Parham, 1996). Discharges to the relatively confined tidal waters of the estuary resulted in depletion of dissolved oxygen, high concentrations of pollutants and a consequent loss of marine and estuarine fauna (Warwick *et al*, 2002).

Since the 1970s there has been a decline in metal-working industries in the Tees Estuary, control of sewage and industrial effluent under legislation by means of consents in which limits are imposed on the volume and concentration of discharges and the introduction of improved treatment measures. These measures have resulted in decreased effluent discharge. Improvements have included the closure of the sewage treatment works at Portrack and Cargo Fleet in 1997 and 1998, respectively and transference of the waste to a new treatment plant at Bran Sands on the lower estuary (Warwick *et al*, 2002). The estuary however, continued to receive a considerable volume of effluent in the 1990s (Davies *et al*, 1991; Huntley *et al*, 2002). In addition, sediments are a sink for historical pollutant loads and could potentially be a source of contamination into the water column. Murrey and

Norton (1979) stated that the Tees estuary sediments were among the most heavily metal contaminated in the U.K., along with those in the Tyne and Mersey estuaries. The authors concluded that these industrialized estuaries could contain concentrations of metals up to 500 times greater than those sediments from the least contaminated estuaries and ports. The Tees estuary was stated to have highly elevated concentrations of zinc (Zn), copper (Cu), lead (Pb), cadmium (Cd), chromium (Cr) and mercury (Hg) compared to less industrialized areas such as the River Bure and Yare in Great Yarmouth. Davies *et al* (1991) reported an overall decrease in the amount of heavy metal contaminants found in the Tees Estuary sediments, although decreases in concentrations of Pb, Cd, manganese (Mn) and nickel (Ni) were only slight. There were more significant decreases in the amount of Zn, Cu and Cr. The metal concentrations decrease in the order Zn >Mn, Pb >Cu >Cr >Ni >Cd. Mean concentrations for some metals appeared low in the Tees Estuary sediments in comparison to highly contaminated sediments of other industrialized regions such as the River Rhine and Weser Estuary in Germany, but metal concentrations in the Tees estuary were similar to the concentrations in these highly polluted regions. Jones and Turki (1997) found decreased Pb and Zn levels in the surface sediments of the Tees Estuary since the 1970s, although the metal concentrations in surface sediments still exceeded background levels with peak values occurring in the upper and middle reaches of the estuary. The background levels with which metal concentrations in the sediment from the Tees Estuary were compared were average metal concentrations in shale published in 1961 and an extensive British Geological Survey data-set of median metal concentrations of North-East England stream sediment (Jones and Turki, 1997).

Sections 1.2.1 and 1.2.2. provide the concentrations of heavy metals in the surrounding environment of the Tees Estuary during the 1990s. The purpose is to provide an understanding of the levels of heavy metal to which the biota have been exposed.

1.2.1. Heavy metal concentrations in solution of the Tees Estuary

Heavy metal concentrations in the water body of the Tees Estuary may potentially be taken up by invertebrates and fish living in the water and by predators consuming these species.

Measurements of heavy metal concentrations in solution are therefore required in order to determine the concentrations available for uptake by the biota. The influence of heavy metal concentrations in solution on concentrations in the invertebrates and the fish will be assessed in Chapter 4 by comparing annual changes in metal concentrations in solution and in the invertebrates and fish.

Metal concentrations in solution in the Tees Estuary were measured during a survey that was initiated to monitor the effects of the construction of the Tees Barrage on the water quality in the main Tees river channel. The survey began in 1990 and ceased at the end of 1997, in accordance with the agreed monitoring programme (Evans *et al*, 1997). Water samples were collected annually from eight stations in the main river channel. Samples were taken on 5 dates during the year (March, May, July, August and October). The tide times were not strictly comparable between years, although from 1994 onwards sampling usually took place at high water of a neap tide. Whilst there have been year to year fluctuations in the maximum concentrations of dissolved metals recorded annually there was a general trend of decreasing concentrations by 1997 (Table 1.1). To obtain an indication of annual average levels of dissolved metals, the maxima recorded for each metal on each different sampling date have been examined and median levels presented in Table 1.2. There was a general trend of decreasing concentrations of Zn, Cu and Cr by 1997, whilst Pb, Cd and Hg concentrations remained relatively constant.

Table 1.1 Annual maximum concentrations of dissolved metals (µg/l) recorded in surface waters of the main Tees river channel (Developed from data provided by Evan *et al*, 1997 as part of the Tioxide Directive monitoring programme)

	Zn	Cu	Pb	Cd	Cr	Hg
1990	74	45	6	0.8	27	0.53
1991	348	20	52	2.0	108	ND
1992	130	860	5	2.0	77	0.26
1993	110	8.6	2.2	0.24	2.8	0.08
1994	38	*	<10	ND	11.4	ND
1995	48	*	2.74	0.22	7.32	<0.01
1996	32	26.8	1.09	0.22	3.14	0.04
1997	30	6.3	1.20	<0.25	4.8	< 0.02

* Results withdrawn by NRA because of uncertainties in analyses revealed by quality control checks, ND Not detected

Table 1.2 Median concentrations of dissolved metals (μg/l) recorded in surface waters of the main Tees river channel (Developed from data provided by Evan *et al*, 1997 as part of the Tioxide Directive monitoring programme)

	No. of sampling occasions	Month	Zn	Cu	Pb	Cd	Cr	Hg
1990	8	5	32.5	6.5	2.5	0.25	8	0.2
1991	12	10	51.5	9	20.5	0.85	28	ND
1992	9	6	47	18	2	0.19	3	0.14
1993	4	4	20	2.6	< 1	< 0.05	< 1	<0.02
1994	3	3	<20	*	< 1	ND	10.2	ND
1995	5	5	28	*	1.38	0.053	< 1	0.04
1996	4	4	6.9	3.3	< 1	<0.05	1.9	0.02
1997	5	5	12	2.0	< 2.5	<0.25	3.9	< 0.02

* Results withdrawn by NRA because of uncertainties in analyses revealed by quality control checks, ND Not detected

1.2.2. Heavy metal concentrations in the sediment of the Tees Estuary

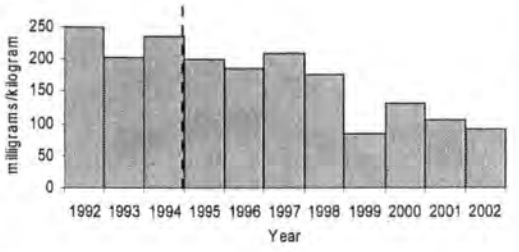
The sediment is a sink for historical and present heavy metal concentrations released into the Tees Estuary (Davies *et al*, 1991). These heavy metal concentrations present in the sediment may potentially be taken up by invertebrates and fish living in the water and by predators consuming these species. Measurements of heavy metal concentrations in sediment are therefore required in order to determine the concentrations available for uptake by the biota. The influence of heavy metal concentrations in the sediment on concentrations in the invertebrates and the fish will be assessed in Chapter 4 by comparing annual changes in metal concentrations in the sediment and in the invertebrates and fish.

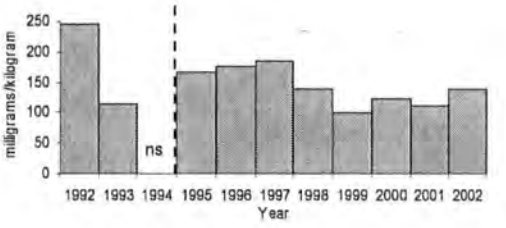
Heavy metal concentrations in subtidal sediments were monitored by the Environment Agency (EA) near the edge of Seal Sands in Greatham Channel and Seaton Channel, between 1992 and 2002 (Figure 1.1 a-g) (Huntley *et al*, 2002). The year of the construction of the Tees Barrage is shown by the dotted line. Datum was collected from one sampling point in Greatham Creek and data were collected from three sampling points in Seaton Channel. There have been year to year fluctuations in sediment metal concentrations in both Seaton Channel and Greatham Channel but most metal concentrations have decreased slightly since 1992, with the exceptions

of Arsenic (As) and Cd. Arsenic has remained relatively constant in both channels since 1995. Cd has increased since 1995, although concentrations were lower in 2002 in Seaton Channel.

Seaton Channel **Greatham Channel**

a)





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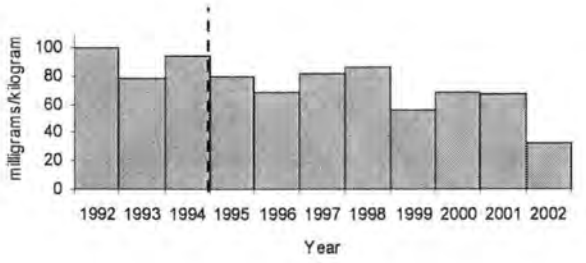

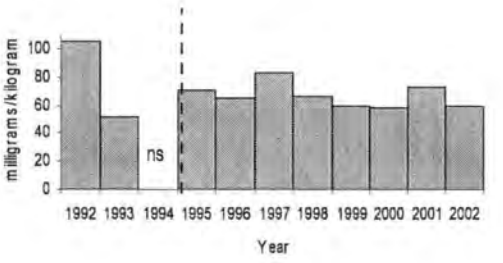

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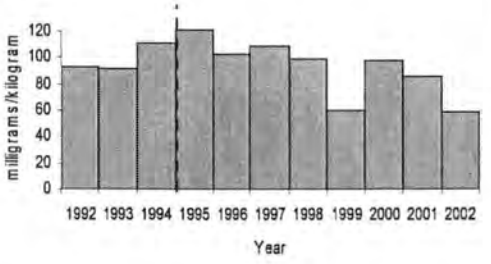

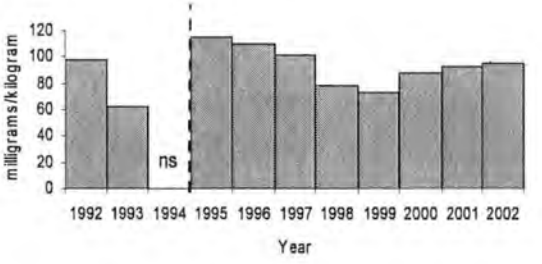

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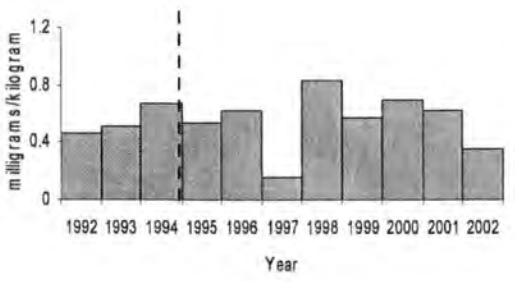

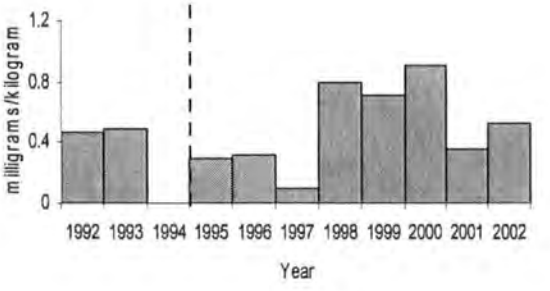

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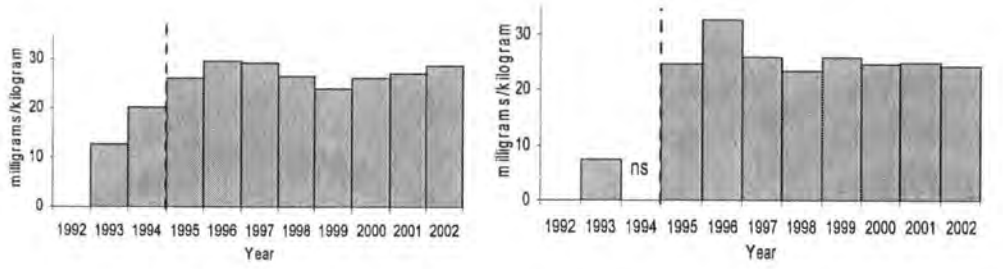

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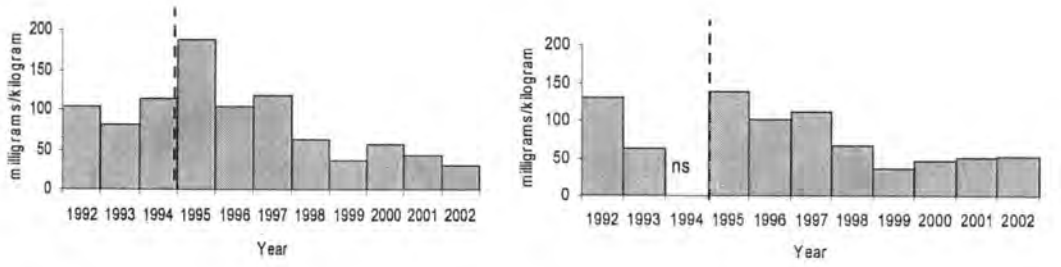

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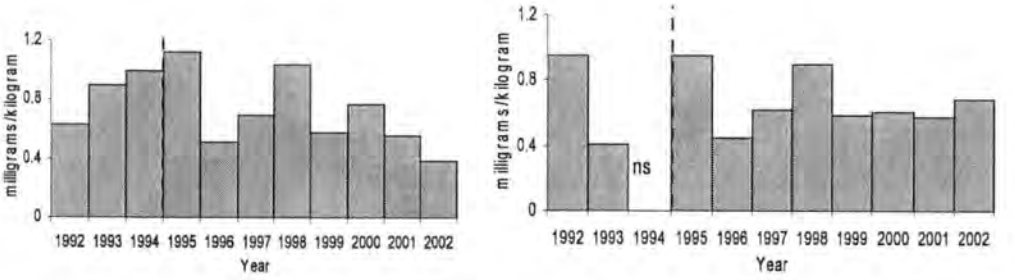


ns = no samples taken nd = not detected

Figure 1.1. Metal concentrations in subtidal sediments from two sites close to Seal Sands mudflats, 1992 to 2002 a) Zn b) Cu c) Pb d) Cd e) As f) Cr g) Hg (Developed from data provided by Huntley *et al*, 2002 as part of the Tioxide Directive monitoring programme) (The dashed line indicates the year of construction of the Tees Barrage).

1.3. BIOTA OF THE TEES ESTUARY

The Tees Estuary provided a range of estuarine habitats, including approximately 2400 ha of mudflats before it became industrialised. Industrialisation adversely affected the organisms of the Tees Estuary due to habitat loss through land reclamation (Appendix A), disturbance from human activities and discharge of pollutants. There has been a decline or disappearance of population numbers in all but the most tolerant estuarine species and a loss of the diversity of estuarine biota. In recent years, there have been reports of increases in some population numbers and species diversity (Huntley *et al*, 2002).

The estuarine invertebrates are an important food source for over-wintering wildfowl and waders. Seal Sands mudflats are a component of the Teesmouth Flats and Marshes which have met the criteria for designation under the terms of the European Directive 79/409/EEC on the Conservation of Wild Birds and for inclusion on the list of Wetlands of International Importance under the Ramsar Convention (J.K. Smith pers. comm). Seal Sands mudflats have been designated a Site of Special Scientific Interest (SSSI) due to their importance to large numbers of migratory wildfowl (c. 4,000) and wading birds (c. 24,000), especially during the winter months

The estuarine food web is a complex system of inter-relationships between species (McLusky and Elliott, 2004). The trophic links within the food web provide important pathways for energy transfer, but also for the transfer of metals. This section describes the ecology and changes in population numbers of key primary, secondary and tertiary consumer species within the estuarine food web. This will provide the background for understanding the transfer of metal concentrations through the top trophic levels of the estuarine food chain.

1.3.1. Benthic invertebrate numbers of the Tees estuary

This section examines the availability and diversity of benthic invertebrate species that are representative of prey consumed by fish, in particular, the ragworm, *Nereis diversicolor,* the mudsnail, *Hydrobia ulvae* and the amphipod crustacean, *Corophium volutator.*

Species diversity and abundance of the benthic populations of macrofauna increased between 1979 and 1985 with a penetration of marine fauna further into the estuary and an increase in abundance in the middle reaches (Shillabeer and Tapp, 1989). A study conducted in 1985-1990, reported the presence of marine fauna in upstream stretches of the River Tees (Tapp *et al*, 1993). The biodiversity of the macrofauna of the Tees Estuary increased between 1979 and 1991, although the number of species present tended to fluctuate annually. There was also an increase in the numbers of individual species. Shillabeer and Tapp (1989) concluded that these changes represented strong evidence of a decline in pollution levels within the estuary. There was a sustained increase in macrobenthos species in the inner estuary coinciding with the construction of the Tees Barrage in 1994 and commissioned in 1995 (Warwick *et al*, 2002).

The abundance and distribution of benthic invertebrate species available for consumption by crabs and fish in the Tees Estuary can be examined from monitoring studies conducted on Seal Sands. Monitoring studies were conducted on this intertidal area at the mouth of the estuary, as a part of the Tees Barrage Monitoring Programme prior and subsequent to commissioning of the Tees Barrage in 1995 (Evans *et al*, 2000; Huntley *et al*, 2002). Overall densities of these three invertebrate species on Seal Sands declined between 1990 and 2002, although there were some increases in numbers in individual years with all three species (Table 1.3).

Table 1.3. The annual changes of densities of the ragworm, *Nereis diversicolor*, the mudsnail, *Hydrobia ulvae* and the amphipod crustacean, *Corophium volutator* on Seal Sands, 1995-2002 (Evans *et al*, 2000; Huntley *et al*, 2002).

	Nereis diversicolor	*Hydrobia ulvae*	*Corophium volutator*
1995	- ve	+ ve	+ ve
1996	- ve	- ve	- ve
1997	- ve	+ ve	+ ve
1998	- ve, almost nil	+ ve	- ve
1999	- ve, almost nil	- ve	- ve
2000	+ ve, small recovery	+ ve	+ ve, approx. half of 1995 population numbers
2001	- ve, almost nil	- ve	No change
2002	- ve, lowest levels since 1990	- ve, lowest levels since 1990	- ve, approx one third of 1995 population numbers

1.3.2. Decapod crustacean species of the Tees Estuary

The Environment Agency (EA) began a count of the number of macroinvertebrates and fish in the Hartlepool British Energy Power Station cooling water intake (Appendix C), July 1991, as part of the Tees Barrage Monitoring Programme (Bastreri, 2002). Initially, monitoring was regular with 24 counts in 1992 and 18 counts in 1993. The annual number of counts then declined to 14 in 1999, seven in each of 2000 and 2001 and only six in 2002. This decline in the annual number of counts makes seasonal comparisons difficult but species presence and annual variability can still be observed. Seven macroinvertebrate species were recorded between 1992 and 2002 (Table 1.4).

Table 1.4. Invertebrate species present in the intake water of Hartlepool Power Station, Tees Estuary, 1992-2002 (Bastreri, 2002)

INVERTEBRATES		
Family	**Species Name**	**Common Name**
Palaemonidae	*Pandalus montagui*	Aesops prawn
Crangonidae	*Crangon crangon*	Common shrimp
Cancridae	*Cancer pagurus*	Edible crab
Portunidae	*Liocarcinus depurator*	Swimming crab
Portunidae	*Carcinus maenas*	Shore crab
Sepiolidae	*Sepiola sp*	Cuttlefish
Asteriidae	*Asterias rubens*	Common starfish

The two dominant species counted in the intake water were the decapod crustaceans common shrimp and the shore crab (Table 1.5). This suggests that they would be available for consumption by seals and cormorants feeding in this area. The presence of invertebrates in the seal and cormorant diet will be determined in Chapter 2 and compared with the counts from the Power Station cooling water intake.

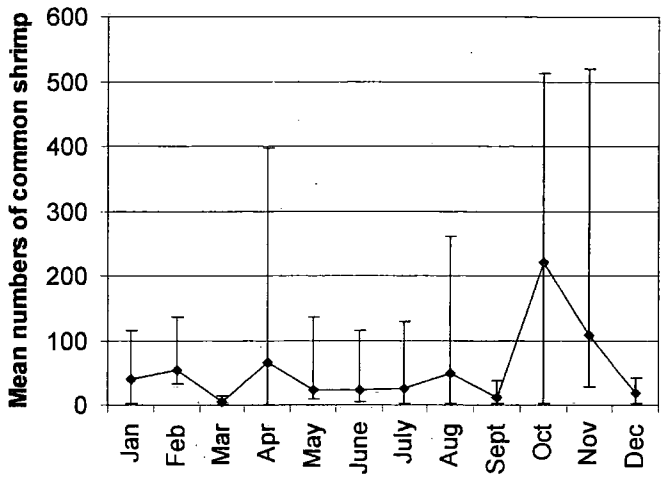
Table 1.5. Numbers of invertebrates counted in the intake water of Hartlepool Power Station, Tees Estuary, 1996-2002 (Bastreri, 2002)

	1996	1997	1998	1999	2000	2001	2002
Aesops prawn	2	10	1	2	0	4	3
Common shrimp	811	923	246	1296	84	367	19
Swimming crab	21	21	20	36	57	6	15
Shore crab	1313	1767	921	1095	1102	579	298
Edible crab	0	0	0	0	0	1	0
Cuttlefish	11	0	0	0	4	0	0
Common starfish	1	1	0	0	0	0	0

The seasonal distribution of the two dominant species, common shrimp and shore crab, counted in the Hartlepool British Energy Power Station cooling water intake between 1996 and 2002 by the EA is shown (Figure 1.2 a and b). This indicates that peak numbers of the common shrimp would be available for predation by seals and cormorants between October and November and peak numbers of the shore crab would be available between June and July. The seasonal consumption of these crustacean species by seals and cormorants will be examined in Chapter 3 and compared with the seasonal distribution shown below.

Shore crab and common shrimp prey on infaunal populations of small bivalves, polychaetes and crustacean (Elliott and Hemingway, 2002). Common shrimp may reduce the population of *Corophium volutator* in estuaries by over 50%. They are also significant predators of the smallest size plaice.

a)



b)

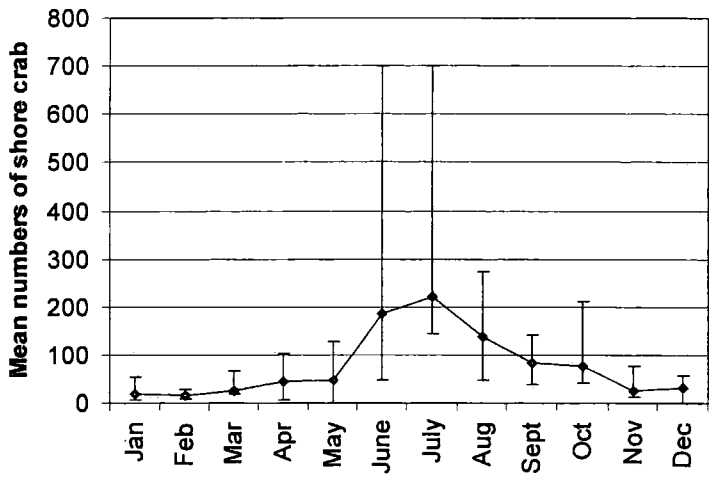


Figure 1.2. a and b. Mean and range of seasonal abundance for two crustacean species counted in samples from the intake water screens of Hartlepool Power Station, Tees Estuary, 1992-2002 a) common shrimp b) shore crab (Developed from raw data provided by D. Bastreri, Environment Agency)

1.3.3. Fish Species of the Tees Estuary

Estuaries naturally support large numbers of fish (Elliott and Dewailly, 1995). Some fish are long-term estuarine residents, whilst the majority use estuaries as a nursery, for overwintering or as migration routes. The number of fish species present in the central section of the Tees estuary from Middlesbrough to Portrack increased between 1984/85 and 1990 to 1994 (Parham, 1996). The Environment Agency (EA) has monitored fish numbers in the Hartlepool British Energy Power Station cooling water intake since July 1991. Forty-one fish species were recorded between 1992 and 2002 (Table 1.6).

Table 1.6. List of fish species counted in intake water screens of Hartlepool Power Station, Tees Estuary, 1992-2002 (Data provided by D. Bastreri, Environment Agency, 2002)

Family	Species Name	Common Name
Petromyzonidae	*Lampetra fluviatilis*	River lamprey
Anguillidae	*Anguilla anguilla*	Eel
Clupeidae	*Clupea harengus*	Herring
	Sprattus sprattus	Sprat
Salmonidae	*Salmo trutta*	Sea trout
	Salmo salar	Salmon
Lophiidae	*Lophius piscatorius*	Angler fish
Gadidae	*Ciliata mustela*	Five-bearded rockling
	Gadus morhua	Cod
	Merlangius merlangus	Whiting
	Pollachius virens	Saithe
	Trisopterus esmarkii	Norway pout
Atherinidae	*Atherina presbyter*	Sand smelt
Gasterosteidae	*Gasterosteus aculeatus*	Three-spined stickleback
	Spinachia spinachia	Fifteen-spined stickleback
Syngnathidae	*Syngnathus rostellatus*	Nilssons pipefish
	Syngnathus acus	Greater pipefish
Triglidae	*Eutrigla gurnardus*	Grey gurnard
	Aspitrigla cuculus	Red gurnard
Cottidae	*Myoxocephalus scorpius*	Bullrout
	Taurulus bubalis	Long-spined sea scorpion
Agonidae	*Agonos cataphractus*	Pogge
Cyclopteridae	*Cyclopterus lumpus*	Lumpsucker
	Liparis montagui	Montagu's sea snail
Serranidae	*Dicentrarchus labrax*	Sea bass
Carangidae	*Trachurus trachurus*	Scad
Trachinidae	*Trachinus vipera*	Lesser weever
Bleniidae	*Blennius pholis*	Shanny
Zoarcidae	*Zoarces viviparus*	Eelpout
Pholididae	*Pholis gunnellus*	Butterfish
Ammodytidae	*Ammodytes tobianus*	Lesser sandeel
	Hyperoplus lanceolatus	Greater sandeel
Callionymidae	*Callionymus lyra*	Dragonet
Gobiidae	*Pomatoschistus minutus*	Sand goby
	Aphia minuta	Transparent goby
Scombridae	*Scomber scombrus*	Mackerel
Bothidae	*Scophthalmus rhombus*	Brill
Pleuronectidae	*Limanda limanda*	Dab
	Platichthys flesus	Flounder
	Pleuronectes platessa	Plaice
Soleidae	*Solea solea*	Sole

The counts of fish in the Hartlepool Power station by the EA are a useful source of information for the species found in the Tees Estuary and distributions of species. The numbers quoted are collected during a particular time period on a set day so they do not represent the total fish present and seasonal and annual comparisons of fish numbers should be reviewed with caution as the regularity of counts has decreased in the latter years. The maximum diversity of fish species recorded in any one year in the Tees Estuary was 32 species in 1994 (Table 1.7). In subsequent years the number of fish species counted was less but this may be due to the less frequent counts made rather than there being less species actually present in the Tees Estuary.

Table 1.7. Percentages and total number of the main fish species counted in the intake water screens of Hartlepool Power Station, Tees Estuary, 1992-2002 (Calculated from raw data provided by D. Bastreri, Environment Agency, 2002)

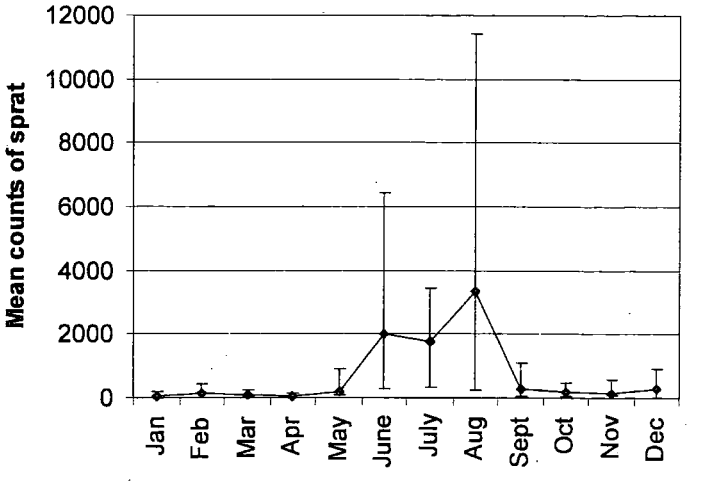
Year	% herring sprat	Total no. of herring sprat	% whiting cod saithe	Total no. of whiting cod, saithe	% flounder plaice, dab	Total no. of flounder plaice dab	Total % of these main fish species	No. of fish species recorded
2002	93.84	3378	0.79	37	1.71	73	96.34	15
2001	93.79	4176	3.53	190	2.07	103	98.39	14
2000	96.97	10999	1.64	121	1.03	89	99.64	17
1999	97.00	22257	1.38	490	1.23	423	99.63	19
1998	89.46	7285	5.47	449	4.02	332	98.97	20
1997	94.38	13557	1.29	185	3.27	456	98.94	24
1996	96.40	15231	2.81	447	0.59	100	99.11	28
1995	96.68	124831	6.76	9654	1.26	2142	99.70	27
1994	97.98	11597	1.10	393	0.95	343	99.30	32
1993	92.51	27379	4.88	1356	1.69	453	99.09	29
1992	97.63	17046	1.87	320	0.64	73	99.14	27

There are large seasonal and annual differences in the total quantity of fish and the counts for each fish species (Figure 1.3.a-d). In each year the total number of fish counted in the estuary peaks in the summer. This reflects the summer migration of sprat, and herring into the estuary (Figure 1.3.a-b). The clupeids, sprat and, to a lesser extent, herring were numerically the most dominant species in the Tees Estuary (Table 1.7).

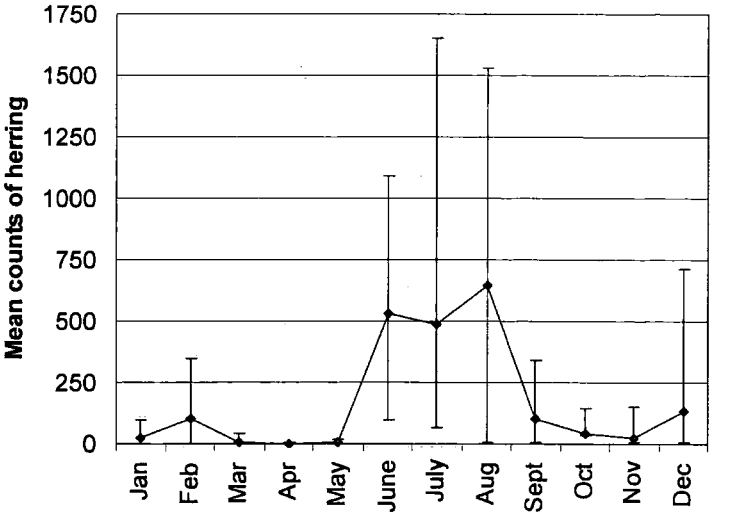
The numbers of gadids, whiting, cod and saithe and the pleuronectids, flounder, plaice and dab counted were considerably lower than sprat and herring but were higher than other species counted (Table 1.7). Whiting and cod numbers peaked during the winter months (Figure 1.3 c). Saithe numbers tended to peak in the summer, with a smaller peak in the winter. Flounder are estuarine residents, whilst plaice and dab are migrants. Pleuronectids appear in relatively small numbers in these counts. The percentage of the total population represented by flatfish was 0.26% in 1995 to 4.02% in 1998 (Table 1.7). These benthic fish are probably under-represented by sampling at the Hartlepool Power Station as the cooling water intake is located in the water column and hence less likely to uptake benthic fish than pelagic fish. Trawl data would have provided a more reliable estimate of flatfish abundance but this was not regularly conducted on the Tees Estuary. In most years flounder numbers peaked in September to October but in 1999 there was a peak in June to July (Figure 1.3.d). Plaice numbers peaked in various months between July and September. Dab peaked during October to November in most years.

The percentage of the total population represented by lesser weever counts varied between 0.11% in 1999 and 0.76% in 2001. The percentage of the total population represented by lesser sandeel counts was 0.003% in 1999 to 0.01% in 1998. These counts from the intake water may under-represent the actual percentage of lesser weever and lesser sandeel in the Tees Estuary since they have a benthic lifestyle.

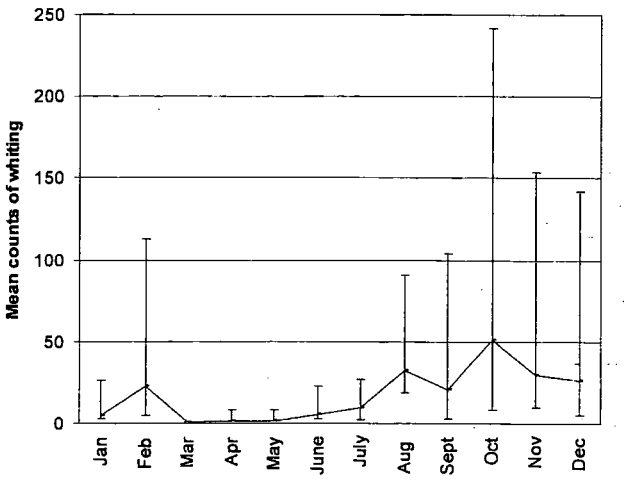
a)


Mean counts of sprat
12000
10000
8000
6000
4000
2000
0
Jan
Feb
Mar
Apr
May
June
July
Aug
Sept
Oct
Nov
Dec

b)


Mean counts of herring
1750
1500
1250
1000
750
500
250
0
Jan
Feb
Mar
Apr
May
June
July
Aug
Sept
Oct
Nov
Dec

c)



d)

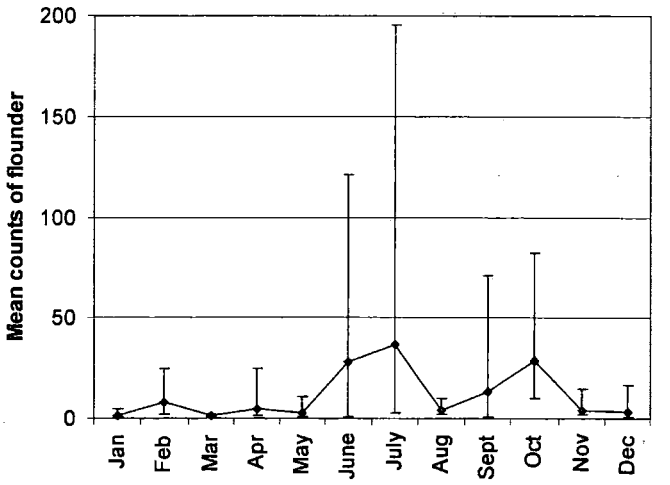


Figure 1.3.a-d. Seasonal changes in abundance (mean and range) of fish species counted in intake water screens of Hartlepool Power Station, Tees Estuary, 1992-2002 a) sprat b) herring c) whiting d) flounder (Calculated from raw data provided by D. Bastreri, Environment Agency, 2002)

Some British estuarine/marine fish species have specific dietary preferences, but most are generalists and thus produce a complex food web (Elliott and Hemingway, 2002). Marshall (1995) analysed the feeding strategy of fish in the Humber estuary and found that all

species, with the exception of sprat, herring, brill and small pogge, had opportunistic diets. There was little evidence of niche overlap however, between species and size classes. This indicates that there is resource partitioning or an over-abundance of prey. Small, epibenthic crustaceans such as amphipods, shrimps, mysids and decapod crabs form an important link between the benthos and fish in the food webs of a number of European estuaries (Elliott and Hemingway, 2002). The occurrence of prey items more likely to be intertidal than subtidal in the diet of many fish species, including *Nereis*, *Nephtys*, *Arenicola*, *Corophium* and bivalve molluscs, such as *Cerastoderma* and *Macoma*, indicated that sole, stickleback, plaice, flounder, lesser weever, eel, brill and turbot, *Scopthalmus maximus* from the Humber estuary tend to use intertidal areas to feed (Marshall, 1995). Plaice were the dominant species caught in intertidal sampling but other species caught included saithe, whiting, cod, herring and sprat. Intertidal areas are well defined as juvenile fish feeding areas (Elliott and Hemingway, 2002). Many dermersal fish are opportunistic feeders and prey choice will reflect the distribution of infaunal species in the area. *Crangon* has been shown to be a dominant food item in the food web of many estuaries such as the Forth and Tagus but this is not the case in the Humber estuary despite a high abundance of the species. In the Humber estuary it is only dominant in the diet of large flounder, large pogge, sea snail, brill and turbot.

Intertidal fish fauna of the Forth estuary, Scotland were found to consume a wide variety of prey taxa (nematodes, oligochaetes, polychaetes, crustaceans and molluscs), encompassing a variety of functional prey groups (benthic-epibenthic-pelagic; errant-sedentary; macrofaunal-meiofaunal and cropped items) (Bryson, 1997). The estimated percentage of total dietary intake by six of the fish species included in this study is presented in Table 1.8 (Falconer *et al*, 1983). This data is a summary of seven studies of the feeding habits of estuarine fish with five of the studies being conducted in Scottish waters, one on the west coast of Britain and the other in coastal waters of Northern Europe.

Table 1.8. Percentage total dietary intake by six fish species (Falconer *et al*, 1983).

Species	Echinoderms	Flatfish	Crustaceans	Polychaetes	Molluscs	Roundfish	Zooplankton
Plaice	30	-	20	20	20	10	-
Cod	5	5	15	10	10	60	-
Saithe	-	-	20	-	-	80	-
Whiting	-	5	10	15	15	70	-
Sprat	-	-	-	-	-	-	100
Herring	-	-	-	-	-	-	100

Elliott and Dewailly (1995) created a total of 29 functional guilds to describe biological characteristics of organisms. The guilds incorporate feeding preferences, reproductive type, substratum preferences (for benthic fish) and position within the water column (vertical preference). The functional guilds of nine dominant fish species in the Tees Estuary, flounder, plaice, whiting, cod, saithe, sprat, herring, lesser weever and lesser sandeel are shown in Table 1.9.

Table 1.9. Guild characteristics of nine fish species commonly found in the Tees Estuary, based on classification by Elliott and Dewailly, 1995

	Type	Habitat	Bottom	Food	Reproductive
Sprat	MS	P	/	P	Op
Herring	MJ	P	/	IF	Ob
Whiting	MJ	D	F	IF	Ob
Saithe	MA	D	R	IF	Op
Cod	MJ	D	F	IF	Op
Flounder	ER	B	F	IF	Op
Plaice	MJ	B	F	I	Op
Lesser weever	MA	B	F	IF	Op
Lesser sandeel	ER	B	S	P	Ob

The designated guilds for these species were:

Type (Ecology)

ER – Truly estuarine resident species, which spend their entire lives in the estuary; **MJ** – Marine juvenile migrant species, which use the estuary primarily as a nursery ground, usually spawning and spending much of their adult life at sea but often remaining seasonally to the estuary, **MA** – Marine adventitious visitors, appear irregularly in the estuary but have no apparent estuarine requirements, **MS** – Marine seasonal migrant species, which have regular seasonal visits to the estuary, usually as adults

Habitat (Vertical Preference)

B – Benthic, living in or on the substratum, **P** – Pelagic, living in the main water column, **D** – Dermersal, living in the water layer just above the bed

Bottom (substratum preference)

S – Sandy bottom, for species living solely on sand, **F** – Soft bottom, for species living on sand, mud and/or fine gravel, **R** – Rough Bottom, for species living on rocks, stones and/or pebbles

Food

P – Plankton, **I** – Invertebrates, such as molluscs, crustaceans or insects, **IF** – Invertebrates and fish

Reproductive Guild

Op – species producing pelagic eggs, **Ob** – species producing benthic eggs

Flounder and plaice are benthic fish species predominately feeding over soft substrate (Elliott and Dewailly, 1995). Flounder spawn in coastal waters then move into the estuary as residents. Plaice primarily use estuaries as nursing grounds. The flounder is an opportunist predator, feeding mainly on crustaceans in the mid estuary and molluscs in the outer estuary, as illustrated by studies from the Thames estuary (Jarrah, 1992). There is some evidence of resource partitioning between juvenile and adult flounder. Immature flounder feed primarily on polychaetes, whereas older flounder feed on a more diverse and seasonally variable diet of gammarids, shrimps, annelids and small fish, such as gobies. Flounder in the Humber estuary consume high numbers of amphipods, polychaetes, decapod crustaceans and molluscs (Marshall, 1995). Small flounder consume a high proportion of plant material, mysids and molluscs. Large flounder consume high proportions of decapod crustaceans, brachyuran crustaceans and fish. Large flounder from the Humber estuary specialize in small and medium fish. There was a decrease in diversity with increasing size in plaice from the Humber estuary with large plaice specialising on the cockle *Cerastoderma edule* and polychaetes (Marshall, 1995).

Whiting and cod are migrants (Elliott and Dewailly, 1995). Juveniles tend to occur closer inshore than adults but all lifestages make considerable seasonal migrations. Saithe are estuarine visitors. All three species feed on fish and invertebrates and have a dermersal

lifestyle. Whiting and cod feed over soft substrate whilst saithe predominately feed over rocky substrate. Whiting are the most abundant gadid species in the Tees Estuary. They tend to live in relatively shallow water, come close inshore and are active predators (Wheeler, 1969). The young live mainly inshore feeding on shrimps, young shore crabs, amphipods, gobies and sand eels. With increasing size whiting eat a more diverse diet including more fish, particularly lesser sandeels and sprat but also plaice, sole and young whiting. They also feed on swimming and hermit crabs and occasionally polychaete worms, small squid and gastropod molluscs. Cod in the Humber estuary consume high numbers of mysids, amphipods and decapod crustaceans (Marshall, 1995). They consume smaller proportions of polychaetes, brachyuran crustaceans and fish. The feeding habits of North Sea cod have been extensively investigated (Macer and Easey, 1988). In 0- and I-group cod the dominant prey items are crustaceans, such as shrimps and copepods, but fish comprise an increasingly important part of the diet with age. Lesser sandeels are important fish prey for younger cod, whilst older cod feed predominately on haddock and whiting. Adult cod exhibit cannibalism of young up to three years of age. Whiting in the Humber estuary consume high numbers of mysids and amphipods (Marshall, 1995). Small whiting consumed a relatively large proportion of copepods, medium size whiting consumed a relatively large proportion of molluscs and large whiting consumed relatively large proportions of polychaetes, decapod crustaceans, brachyuran crustaceans and fish. First year saithe feed mostly on copepods, littoral amphipods, molluscs and the fry of cod, saithe, gobies and sand eels (Wheeler, 1969). Immature saithe are common offshore and feed on crustaceans, especially copepods and euphausiids, and on fish, particularly sand eels and young cod. Most gadids in the Tees estuary are juveniles or young adults and so are at the smaller end of their size range.

Clupeids, sprat and herring, are the most numerically abundant fish species of the Tees Estuary. They are pelagic fish, swimming in shoals and are migratory (Wheeler, 1969). Sprat are seasonal migrants, whereas herring tend to use estuaries as nursing grounds (Elliott and Dewailly, 1995). The main migration time into the Tees Estuary is in the summer. Adult sprat tend to spawn out to sea, then the larvae drift inshore and the young of

the year continue to live close to the coast, often in shoals with first year herring. The food of the adult sprat is composed mainly of planktonic crustaceans, especially copepods and mysids. Juvenile herring are mainly planktonic feeders but as adults they feed on a variety of invertebrates and fish including crustaceans such as copepods, amphipods, euphausiids and mysid shrimps, other invertebrates, including arrow-worms, ctenophores and pteropods, and small fish, particularly sand eels, gobies, young whiting, herring and flatfish. Herring from the Humber estuary fed predominately on mysid shrimps (Marshall, 1995).

Lesser weever are benthic fish, burrowing in the sandy bottom (Lythgoe and Lythgoe, 1971). Lesser weever in the Humber estuary consumed high numbers of mysids, amphipods and decapod crustaceans (Marshall, 1995). They consume smaller proportions of polychaetes, brachyuran crustaceans and in the adults, fish. Lesser sandeels are shoaling fish and planktonic feeders throughout their lifespan but spend much of the time buried in the sand in shallow waters with depths from 30m.

Dragonet is a benthic fish living in shallow water over sand and mud (Wheeler, 1978). In the southern North Sea growth appears to be restricted to the period May/June to October (Hall *et al*, 1998). Peak feeding occurs during these warmer months. Dragonet in the Humber estuary consumed high numbers of molluscs, decapod crustaceans, amphipods and brachyuran crustaceans throughout their lifespan (Marshall, 1995).

1.3.4. Cormorant numbers in the Tees Estuary

A large number of the North Atlantic race of the cormorant, *Phalacrocorax carbo carbo* inhabit Britain. The birds breed almost exclusively in small, widely distributed, coastal colonies of about 10-200 pairs (Lloyd *et al*, 1991). In England there is a population of 3100 breeding pairs, with a further 1700 breeding pairs in Wales (Mitchell *et al*, 2004). Persecution by man caused a large decline in cormorant numbers during the nineteenth century. Cormorant numbers have now recovered. They are protected under the Wildlife and Countryside Act, 1981, although they can be killed under license in the event of damage to fisheries.

In addition to cormorants breeding in Britain there has also been an increase in the numbers of cormorants wintering in Britain by 74% over the past 20-30 years with the population estimated to have reached at least 18,700 birds in 1990/91 (Kirby *et al*, 1995). The increase in winter population appears to be a combination of an increase in the absolute numbers of birds breeding in Britain and Ireland and also an expansion in the continental subspecies *P. c. sinensis* with some birds migrating to Britain. Roughly 5-10% of the British wintering population are *P. c. sinensis*. Cormorant numbers in the Tees Estuary have increased in recent years (Figure 1.4). From the mid to late 1970s the average maximum count was 75 birds, whereas the average maximum count between 1990 and 1998 was 392 birds and the highest average maximum count of birds was 860 in 1999 (Bell, 1996, Armstrong, 1999).

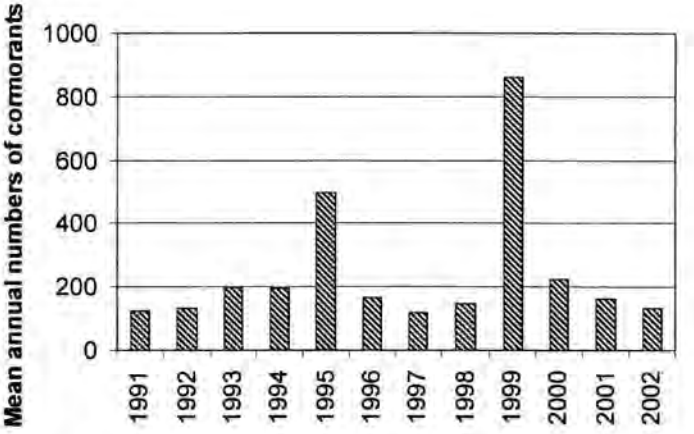


Figure 1.4. Annual numbers of cormorants averaged over all months in the Tees Estuary, 1991-2002 (Calculated from Little and Iceton, 1991-2002).

Cormorants generally occupy their breeding colonies from mid-March to mid-September, with egg-laying in late April to early May (Cramp and Simmons, 1977). They disperse widely after the breeding season generally moving south and east in Britain, and many others over-winter on the Atlantic coast of France, Spain and Portugal (Hagemeijer and Blair, 1997). Higher numbers of roosting cormorants (based on the monthly average) were present during the winter months in the Forth Estuary on the Alloa bridge pillars (1984-1989) (Elliott and Hemingway, 2002). Some of the cormorants roosting in the Tees estuary

are residents, whilst others are winter visitors and numbers vary seasonally with the highest average seasonal count in August to September for the period 1991-2002 (Figure 1.5). The counts took place on the same date each month to ensure uniformity.

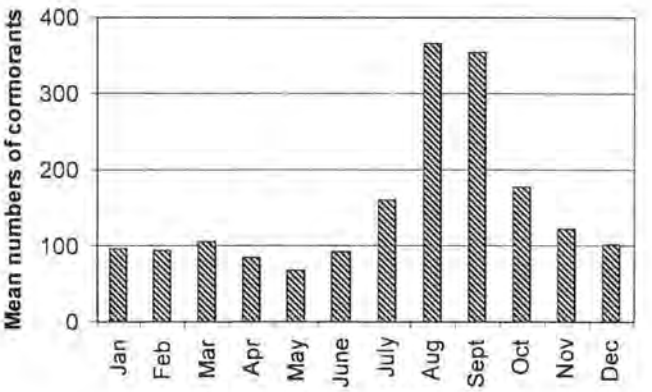


Figure 1.5. Average seasonal numbers of cormorants in the Tees Estuary, 1991-2002 (Calculated from Little and Iceton, 1991-2002).

Cormorants are almost entirely piscivorous (Kirby *et al*, 1996). They tend to be coastal foragers, although recently some have extended their range inland and forage on freshwater fish species, particularly during the winter months. This switch from coastal to inland habitats is likely to be a result of competition, decreased food supplies at the coast and increased food supplies inland with the development of fish farming (Kirby *et al*, 1995). Only 50 to 54% of cormorants are found on the coast in February. The majority of research on cormorants has been conducted in the freshwater environment due to concern from recreational fishermen and fish farmers (Kirby *et al*, 1995). The prey consumed in freshwater and estuarine environments will be considerably different but research conducted in the freshwater environment can still be used to show the opportunistic feeding behaviour of cormorants and the average quantity of food consumed.

1.3.5. Seal numbers in the Tees Estuary

Two species of seal occur on the Tees Estuary, the harbour seal, *Phoca vitulina* and the grey seal, *Halichoerus grypus*. Harbour seals are distributed around the coasts of the North Atlantic and North Pacific from the subtropics to the Arctic. Harbour seals in Europe belong to a distinct sub-species, *Phoca vitulina vitulina*. Britain holds approximately 40% of the world population of the European sub-species. The Sea Mammal Research Unit (SMRU) carry out an annual survey of harbour seal numbers during the moult in August when they spend the largest proportion of their time on land and are therefore visible to be counted (Sea Mammal Research Unit, 2006). Most regions are surveyed using thermographic, aerial photography to identify seals along the coastline. Conventional photography is used to identify seals in the Wash and visual counts are conducted annually in the Inner Moray Firth by the University of Aberdeen. The counts are a minimum estimate of the population size of harbour seals in UK waters since at any one time some seals are likely to be in the water and numbers hauling out also vary with the state of the tide and the weather (Sea Mammal Research Unit, 2002). The English population of harbour seals in 2005 was approximately 3637 seals, whereas the total British population of harbour seals is approximately 32696 seals with approximately 29059 counted on the coastline of Scotland (Sea Mammal Research Unit, 2006). The Lincolnshire and Norfolk coastline in the east of England holds 95% of the English population of harbour seals. The British population of harbour seals was affected by the phocine distemper virus (PDV) epidemic in 1988 and 2002. In 1988, the numbers of harbour seals in the Wash on the east coast of England declined by approximately 50% due to the PDV epidemic (Sea Mammal Research Unit, 2006). Prior to this numbers increased again until the PDV epidemic in 2002. Mortality was lower than in 1988 at approximately 22%.

About 38% of the world population of grey seals is found in Britain and over 90% of British grey seals breed in Scotland, the majority in the Hebrides and in Orkney (Sea Mammal Research Unit, 2002). In 2001, a total British population of 130000 grey seals was recorded, along with an estimated 42000 grey seal pups born in Britain. Grey seals are

rarely affected by the phocine distemper virus and the British population is increasing. This is demonstrated by the increase in grey seal pup numbers at Donna Nook in Lincolnshire, which is south of the Tees Estuary on the English east coast. In 1981 34 grey seal pups were counted, by 1989 this had increased to 94 pups and by 2003 the number of pups counted had increased to 792 (Lincolnshire Wildlife Trust pers. comm.).

The Tees seal colony of both harbour and grey seals has become re-established on the Seal Sands National Nature Reserve in the Tees Estuary. Amateur naturalists recorded over 1000 seals hauled-out on Seal Sands during the eighteenth century but seals were not observed at Seal Sands from the early nineteenth century (Parham, 1996). The disappearance of the seals coincided with the most intensive period of land reclamation and industrial development in the Tees Estuary and was probably due to a combination of habitat loss, disturbance, decreasing water quality and consequential reduction of prey availability. The Seal Sands mudflats supported a breeding population of over a thousand harbour seals before industrialisation. The mudflats have been progressively reclaimed for agriculture and then industrial development until only about 10% of the original area remains.

There were sightings of individual seals in the Tees Estuary in the 1960s and 1970s but a regular group hauling-out on Seal Sands was not recorded until the 1980s. A small colony of harbour seals has now re-established and has bred successfully since 1994 (pers. obs.). A smaller group of non-breeding grey seals also haul-out during the summer. Most grey seals leave Seal Sands during the winter to breed in large colonies on rocky shores to the north, whilst a few non-breeding grey seals, particularly juveniles, remain at Seal Sands.

The Tees seals research programme was initiated in the autumn of 1988 to monitor the status of the seal colony on the Tees Estuary and their ability to live alongside industry. Population numbers of harbour seal and grey seal have gradually increased, as they have in other parts of Britain. Initially, recruitment was mainly a result of immigration but 25 pups have been successfully weaned since 1994 and some of these pups may have remained in the area. There is some post-weaning dispersal, but seals are mainly faithful to haul-out

sites (Tollit, 1996). Intensive observations have been made during the harbour seal pupping season of mid-June to early September, 1989-2003 at the Seal Sands mudflats during low tide. The number of seals hauling out varied daily and seasonally as a result of weather conditions, and occasionally disturbance. Maximum counts were recorded on sunny, still days when the seals bask to restore valuable energy stores. A study of harbour seal populations in the Straits of Georgia and Puget Sound found a correlation between the numbers of seals hauling out and weather conditions, due to an energy trade-off between effort and thermal regulation (Olesiuk *et al,* 1990). The maximum number of harbour seals observed on Seal Sands on any one day has increased from 23 in 1989 to 71 in 2001 and 2002. There was a decline in 2003 to 58 seals (Figure 1.6). The maximum number of grey seals observed on Seal Sands mudflats on any one day was 18 in 1989, increasing to 30 by 2002.

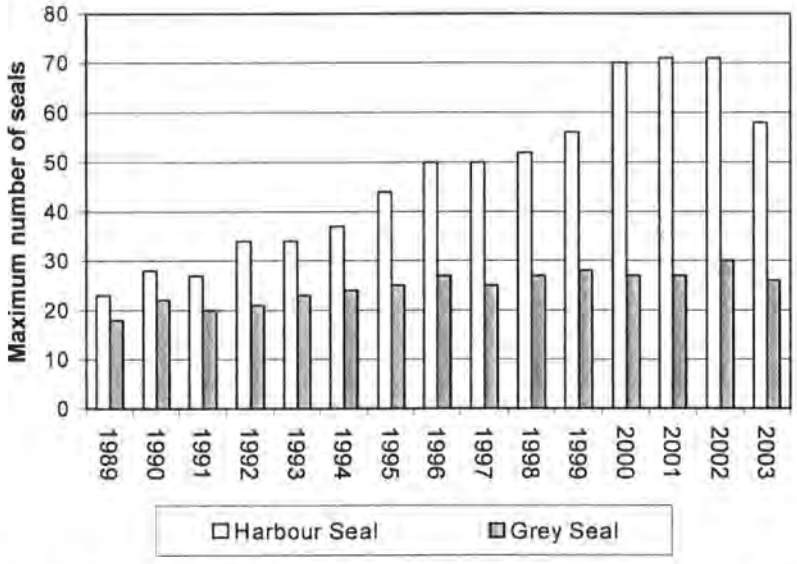


Figure 1.6. Maximum counts of harbour and grey seals in the Tees Estuary, 1989-2003 (Calculated from raw data provided by R. Smurthwaite, INCA, 2002; J. Gibson, INCA, 2003)

The number of harbour seal pups born and surviving has increased since monitoring began in 1989 (Smurthwaite, 1996). Between 1989 and 1993 a single harbour seal pup was born in alternate years. All three pups died within 1 to 5 days of birth. The first two pups born

and successfully weaned were recorded in 1994. The number of pups born each year has gradually increased since, although some have been deserted or died (Figure. 1.8). Four pups were rescued between 1995 and 1997 (Turner, 2003). Three were abandoned at a young age and suffered from malnutrition. The fourth pup was rescued after it had weaned because it was starving. In 1999, five pups were born and four strong and healthy pups survived to weaning. The other pup was stillborn but it was not possible to recover the body for tissue analysis as the mother carried it in her mouth for over a week. Four to six healthy pups were born in 2000 and 2003. All of these pups survived to weaning (Figure 1.7).

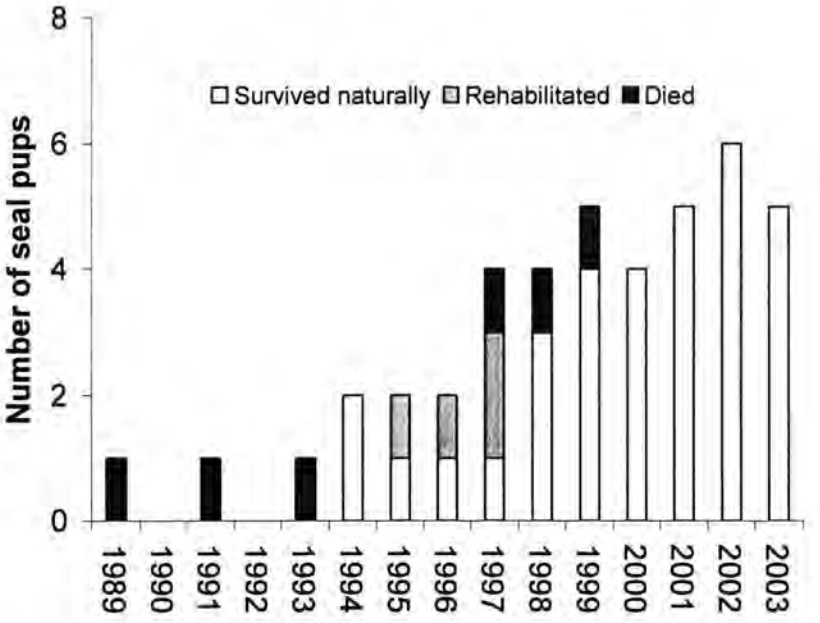


Figure 1.7. Numbers of harbour seal pups born and their fate to weaning in the Tees Estuary, 1989-2003

Birth rate and the survival rate of pups in the Tees Estuary are gradually improving but they still appear relatively low in relation to colony size. According to Reijnders (1982) the reproductive rate of a harbour seal colony considered normal is approximately 20-30% of the population. Estimated first year mortality in the Kattegat and Skaggerak bays of the North Sea bounded by Denmark and Sweden was 33% and pre- and post-weaning pup non-viability rate was less than 10% (De Jong *et al*, 1997). Boulva (1971) reported mortality in harbour seal pups, from Sable Island, Nova Scotia, during the first month of their life to be

in excess of 12%. The high mortality in the Tees Estuary could be reflective of naturally high first year pup mortality for harbour seals, but the small population size makes this difficult to ascertain. The relatively new and small colony may have required time to establish before breeding was successful. Initial recruits may have been outcasts from other colonies due to population pressure and possibly not reproductively viable.

Habitat loss due to land reclamation may have reduced the viability of the colony. The Tees seals haul-out around the low-tide period on Seal Sands mudflats. The mudflats are exposed for at least 4 hours and up to 8 hours and the seals use different mudflats preferentially due to the state of the tides. When the Seal Sands mudflats are submerged by the tide some harbour seals use Greatham Creek as an alternative haul-out site (Appendix C). They have also been observed using haul-out sites further upstream towards the Tees Barrage. The Tees seals are continually finding new haul-out sites so habitat does not appear to be a limiting factor.

Disturbance may adversely affect pup survival as it reduces resting time and uses energy resources. In addition, it may decrease the duration of suckle bouts which influence pup weaning mass (Engelhard *et al,* 2001). Pup weaning mass is positively associated with 1st year survivorship. Engelhard *et al* (2001) did not find a direct effect of human disturbance on the efficiency of lactation in elephant seal, *Mirounga leonine* pups between two areas on the sub-Antarctic Macquarie Island despite one area being remote and the other having a relatively high human presence. Seals scan for potential threats whilst hauled out and this alertness reduces their rest period. Seals haul-out in groups for the benefit of predator detection rather than stable social units (Godsell, 1988; Terhune and Brilliant, 1996). Disturbance causing the seals to enter the water may result in the mother using valuable energy sources needed for lactation or the mother-pup unit becoming separated. Allen *et al* (1984) observed the affects of disturbance, including pedestrians, dogs, aircraft and boats, especially yachts on harbour seal haul-out behaviour at Bolinas Lagoon, California. The proximity of the disturbance had a more significant effect on the seal's reaction than the type of disturbance or the season. The disturbance changed normal seasonal haul-out

patterns and, in severe cases, a change in diurnal to nocturnal haul-out, and hence less energy input per haul-out due to the lack of sunlight, increased site abandonment and a possible increase in pup mortality. The disturbance recorded on Seal Sands between 1989 and 1996 was sporadic throughout the year and generally at a low level. The seals' reaction to potential disturbance was influenced mainly by proximity but they were also disturbed by casting of sails and other sudden movements. The main cause of disturbance was industrial boats involved in temporary construction work in 1989-1990. Disturbance decreased subsequently until 1995 when there were 14 disturbance incidents caused by 25 recreational boats. Disturbance incidents have decreased in recent years with only seven disturbance incidents in 2003.

The low pupping rates and breeding failure may result from inadequate maternal care. The pups that died and those that were abandoned were suffering from malnutrition. Nursing was regularly observed, however, the mothers were often seen trying to encourage their pups to feed and there were frequent displays of mother pup bonding. This suggested that the mothers were not able to supply an adequate quantity of milk rather than that their care was inadequate.

Harbour seals are principally piscivores and opportunistic feeders (Härkönen and Heide-Jørgenson, 1991). Harbour seals consume mainly coastal and estuarine fish species (Prime and Hammond, 1990). Radio tracking studies have shown that harbour seals in the Moray Firth were coastal foragers, feeding within 30 to 60 km of their haul-out site (Tollit, 1996; Thompson *et al*, 1996; Thompson *et al*, 1998). This research suggests that harbour seals hauling out on mudflats in the Tees Estuary will feed within the estuary on a range of coastal and estuarine fish species.

1.4. AIMS AND OBJECTIVES OF THE THESIS

The aim of this thesis was to use a bottom–up approach to estimate the heavy metal uptake by two top predators from the Tees Estuary, harbour seals, *Phoca vitulina* and cormorants, *Phalacrocorax carbo*. The objectives were to determine the seasonal biomass of each species consumed by these predators, to measure the metal concentrations in their prey, decapod Crustacea and fish and to use this data to estimate the seasonal metal uptake by seals and cormorants. This would indicate the level of metal contamination in the top trophic levels of the Tees Estuary and the implications for the condition of these organisms and consequently the estuarine food chain.

The species and size of prey consumed by harbour seals and cormorants was determined from analysis of hard remains in seal faecal samples and cormorant pellets in Chapter 2 and this information was then used to estimate the seasonal biomass of each species consumed by seals and cormorants in Chapter 3. The variation in metal concentrations between prey species was investigated in Chapter 4, as was the affect of season, prey length, prey mass and the interaction between metal species. The bi-monthly biomass of each species consumed was then multiplied by the metal concentrations in each of the species to estimate the metal uptake by seals and cormorants in Chapter 6 and Chapter 7, respectively. The metal concentrations egested in seal faecal samples were measured and they were compared with the metal burden taken up by the seals to estimate retention. Metal concentrations in the whole body of Crustacea and fish were analysed in Chapter 4 to enable metal uptake by predators from consuming the whole body of the prey to be estimated. Chapter 5 measures the metal concentrations in the soft parts and the exoskeleton of Crustacea to assess the affect of including the exoskeleton on the analysis of metal concentrations in the whole body. Predators may not ingest the exoskeleton or if ingested the exoskeleton may not be digested and so stored metal concentrations would not be bioavailable. Metal concentrations in fish liver, muscle and gills were analysed to be able to compare the concentrations in these body tissues with those quoted in published literature.

A bottom-up approach, estimating metal uptake by predators from prey, was used because killing, damaging or disturbing top predators in the Tees Estuary to directly measure metal concentrations in body tissues was regarded as unacceptable. Obtaining carcasses of predators that have died from natural causes is unpredictable and infrequent due to the low mortality rates of these long-lived predators with relatively small populations. Metal concentrations were measured in the carcasses of two adult seals obtained due to natural mortality but statistical analysis could not be conducted and a sample size of two and one of the seals was a grey seal, *Halichoerus grypus* whereas this study concentrates on harbour seals. Indirect methods of studying pollutant loads in top predators were implemented, based upon the approach suggested by Reijnders (1988), that food intake by seals (or other predators) can be used to assess the availability and quality of the food resource and the potential for accumulation of pollutant loads via ingested prey.

Metal discharge levels in the Tees Estuary have declined in recent years (Parham, 1996; Huntley *et al*, 2002) but may persistent in the estuarine sediment and be available to predators via their diet. The uptake and bioaccumulation of metals in marine organisms is of concern for two reasons. Firstly, the adverse effect that these metals may have on population numbers and dynamics (Eisler, 1981). Secondly, some metals have the potential to biomagnify and the highest concentrations then occur in organisms at the top of the trophic chain, such as marine predators (Eisler, 1981). Top predators exist in relatively small numbers, usually have slow reproductive rates, and are correspondingly slow to recover in the event of population declines (Walker, 1990). It is important that metals do not bioaccumulate to concentrations that will have an adverse effect. The population size of the harbour seal colony hauling out within the Tees estuary has been gradually increasing since the 1980s (Smurthwaite, 1996). The number of pups born, however is low in relation to the number of adults present and there have been six pup mortalities and four pups rescued and rehabilitated. This study assesses whether the metals uptake by the adult seals is high and therefore has the potential to adversely affect reproduction. Cormorant diet and metal intake was assessed as a comparison to harbour seal diet (Chapter 3) and metal intake (Chapters 6 and 7), since they are both opportunistic piscivores feeding in the Tees Estuary.

1.5 HYPOTHESES

Chapter 2 and 3 Diet of cormorants and seals

- The numbers of prey species consumed seasonally will be estimated from the presence of skeletal remains in seal faecal samples (Arim and Naya, 2003) and cormorant pellets (Zijlstra and Vaneerden, 1995).
- The length and mass of prey species consumed will be estimated from otolith size (Härkönen, 1986; Tollit, 1996).
- The seasonal diet of cormorants and seals is expected to show significant niche overlap because they are both opportunistic piscivores feeding in the Tees Estuary.

Chapter 4 Metal concentrations in Crustacea and fish

- Metal concentrations in Crustacea and fish body tissues are expected to be significantly different between species (Phillips, 1980; Lawrence and Hemingway, 2003).
- Metal concentrations in Crustacea and fish body tissues are expected to be significantly different between seasons (Phillips, 1980; Lawrence and Hemingway, 2003).
- Metal concentrations in Crustacea and fish body tissues are expected to correlate with body size (Cossa *et al*, 1992; Henry *et al*, 2004).

Chapter 5. Compartmentalization of metals in Crustacea and fish

- There will be differentiation between metal concentrations in body tissues of Crustacea (Chan, 1990; Rainbow, 1990) and fish (Henry *et al*, 2004).

Chapter 6 and 7 Metal concentrations in seals and cormorants

- Uptake of metal concentrations by harbour seals and cormorants can be estimated using the mass of prey consumed and metal concentrations in the body tissues of their prey species (Reijnders, 1988).
- Metal concentrations in seal faeces are expected to be a major output route for metals taken up by seals from their prey species (Mason and MacDonald, 1986).

CHAPTER 2. IDENTIFICATION OF PREY REMAINS FROM PREY SPECIES AND ESTIMATION OF PREY SIZE USING FISH FROM THE TEES ESTUARY

2.1 INTRODUCTION

Analysis of skeletal remains in faeces or regurgitated matter of predators is now the most widely used method of estimating diet (Arim and Naya, 2003). This analysis can be used to identify prey species ingested and to estimate the frequency and size of prey consumption. Skeletal remains can be obtained from faecal samples of seals or regurgitated pellets of cormorants, *Phalacrocorax carbo* collected from their haul-out or roost sites without harm to the predators. Care must be taken to avoid disturbance as this can depress breeding success in seals and may affect the location of where the predators defecate. It is not usually possible to assign the source of each faeces or regurgitated pellet, so bias may occur from differences in season, sex, age and individual prey preference, although bias can be reduced by using a large sample size. Large numbers of seals however, do not necessarily guarantee the presence of seal faecal samples, as they can be voided out to sea. Coastal and estuarine prey species may be over-represented in the seal faeces collected at haul-out sites as fish consumed offshore are more likely to be voided out to sea. Trites and Joy (2005) concluded from Monte Carlo simulations and frequency of occurrence methods that a minimum of 59 faecal samples would identify the principal prey remains occurring in >5% of faeces and 94 faecal samples would allow comparisons of diet over time or regions.

Some carnivorous and piscivorous birds, including cormorants, produce regurgitated pellets containing undigested material. In birds, pellets tend to be more reliable for dietary analysis than faecal samples as the remains are less exposed to erosion in the acidic digestive system. Johnson and Ross (1996) compared the relative importance of pellets and faeces in describing the diet of the double-crested cormorant, *Phalacrocorax auritus*. Ninety percent of fish remains were found in pellets compared to only 10% in faeces, whereas only 4% of the remains in the faeces represented fish that could not be accounted for in pellets. In captive trials to determine pellet production and otolith passage cormorants produced one pellet per day independent of the number of meals or species of fish consumed (Zijlstra and Vaneerden, 1995). Diet was changed to observe whether otoliths of prey consumed several

days previously would be present in regurgitated pellets. Pellets regurgitated at daybreak only contained undigested remains from the previous day. One cormorant in the trials did not eat on one day and it did not regurgitate a pellet the following day. Hard parts found in one pellet are therefore considered to represent the daily diet of an individual whereas faecal samples may only represent a part of the diet.

2.1.1. Prey species identification from skeletal remains

Information regarding bone morphometrics is widely scattered in the literature (Pierce and Boyle, 1991). The University of Aberdeen has established a reference collection of skeletons for up to 80 North Sea fish species (G. Pierce, 2001, pers. comm.). Fish in archaeological studies have been identified from bones (Casteel, 1976). Skeletal parts used for fish identification, include the vertebrae, scales (Wise, 1980), premaxilla (Conroy *et al*, 1993), dentaries, opercles, cleithra (Hansel *et al*, 1988; Scharf *et al*, 1998), pharyngeal teeth and otoliths (Härkönen, 1987). The backbone of a fish comprises 40 to 60 vertebrae. Vertebrae and scales are of limited use in estimating the number of prey consumed due to the difficulty of determining whether several vertebrae or scales in a faeces have originated from the same fish, or from more than one individual (Wise, 1980). In addition, scales are often damaged during their passage through the digestive system, and it is difficult to measure the diameter of small scales in fishes. Some fish species have a high proportion of damaged scales and replacement scales, with atypical dimensions.

The premaxillae are a pair of bones forming the anterior margin of the upper jaw (Conroy *et al*, 1993). Dentaries are paired and are the largest bones of the lower jaw and hold some teeth for many fish species. The opercle is the largest and most dorsal bone of the three bones of the opercular series providing skeletal support for the muscular operculum (Scharf *et al*, 1998). The cleithrum is the ventral most bone of the pectoral girdle in higher teleost fish. Scharf *et al* (1998) assessed the potential value of the cleithra, dentary and opercle for identifying ten marine fish species from the Northwest Atlantic recovered from predator stomachs. There were unique diagnostic features between family taxa for each of the three bones. The cleithra and dentaries, however, were the better tools for distinguishing the

differences between genera within the familes, Gadidae and Clupeidae than opercles, whereas differences between diagnostic features of the bones for two species from the same genus (*Alosa*) were difficult to discern. Diagnostic features of the cleithra and dentaries may therefore be tools for distinguishing between genera but not necessarily for species within the same genus. Pharyngeal teeth are of great value in determining cyprinid species (Wheeler, 1969). They are paired throat bones, located behind the gill arches and partly beneath the pectoral fin girdle. The number of teeth and rows of teeth differ between species, as does their individual shape and form. The teeth can be on one to three rows and in some species one side may have one tooth more than the other side.

Otoliths are the calcareous inner ear bones of teleost fish, found within the labyrinths (sense organs). The paired membranous labyrinths are situated within and at the back of the cranial cavity on either side of the head. The labyrinths contain endolymph and three main bags, each containing an otolith (Figure 2.1).

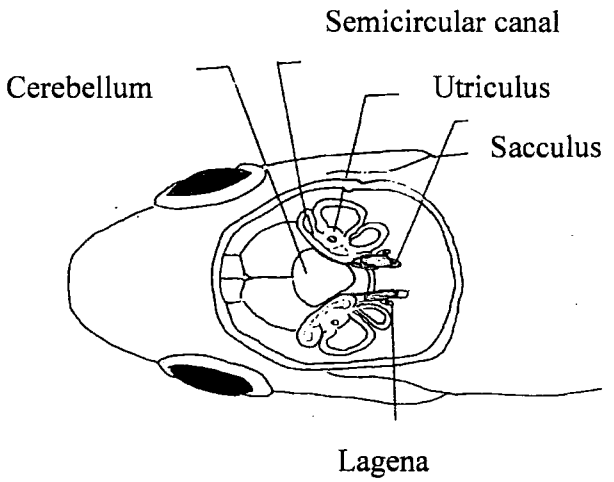


Figure 2.1. Dorsal view of the labyrinth within the fish head (Härkönen, 1986)

Some parts of the inside of the labyrinths, especially the membrane close to the otoliths, are covered with sensory nerve endings which react to pressure and movement of the otoliths enabling the detection of gravity, acceleration, retardation and sound (Härkönen, 1986). There are three otoliths on each side of the head but the sagitta is the main otolith used to identify the fish species consumed, to quantify prey consumption and to estimate prey size

because it has a distinct shape for different fish species, ranging from flat-oval to spindle-shaped structures. The lapillus and asteriscus otoliths are very small and seldom detected.

The general shape, curvature of the margins and the size of the sagitta otolith are important characters for identification (Härkönen, 1986). The pointed end is usually the anterior part, except in gadids where the posterior end is more pointed than the anterior end. The inside of the otolith has a longitudinal structure called the sulcus close to the centre of the otolith. The anterior part of the sulcus is called the ostium and the posterior part is the cauda. The meeting point of the cauda and the ostium is often marked by a shallow or narrow section of the sulcus (Figure 2.2.)

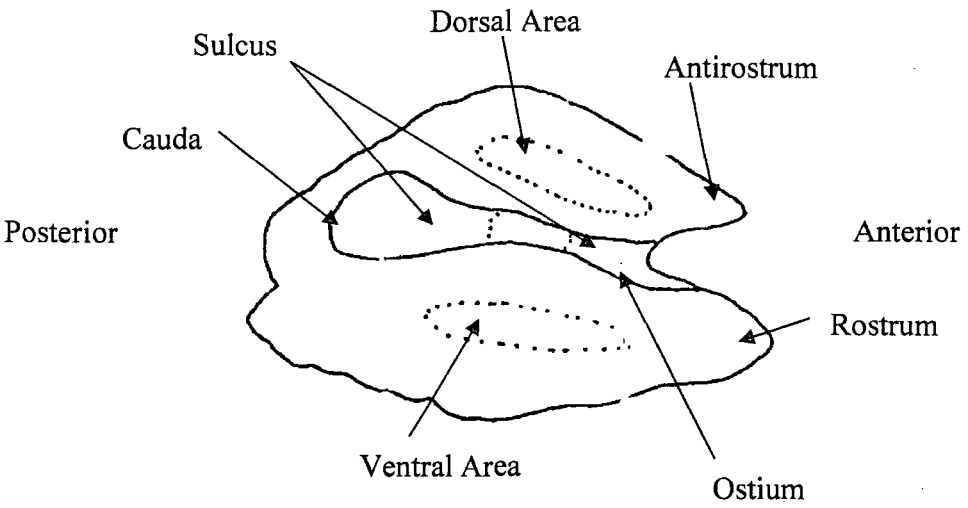


Figure 2.2. The morphology of the inner face of a left otolith (Härkönen, 1986)

The ostium and cauda can both reach the anterior and posterior margins respectively and are called 'open' if they meet the margins and 'closed' if they do not. In some groups the sulcus can be partly filled or completely filled with aragonite of a slightly different composition than the otolith body, this is referred to as 'colliculum'. Dorsal and often ventral of the sulcus is mostly a flat or concave section of the surface of the inside of the otolith. These sections are referred to as 'areas'. There is often a more or less distinct rostrum protruding from the anterior ventral part of the otolith body and the size and shape of this rostrum is important for species identification. Dorsal to the rostrum is the variably developed,

protruding or bulging antirostrum, although the antirostrum can be absent from some species. The inside of the otolith can be convex, flat or concave, whereas the outside is relatively even and often lacking in discrete structures for most species, except some groups such as the gadids. In gadids the surface of the outside is often strongly and discretely lobed.

Captive feeding trials have compared the accuracy of identifying fish species from skeletal remains in the faeces of grey seals (Prime and Hammond, 1987) and harbour seals, *Phoca vitulina* (Cottrell *et al*, 1996). In both studies, fish vertebrae were the most frequent remains recovered, with otoliths and fish eye lenses also present in the majority of samples. In the study by Cottrell *et al* (1996) vertebrae constituted 66% and otoliths 17% of all taxon-specific hard parts identified. Vertebrae were the most important structures for determining the presence or absence of herring, *Clupea harengus,* smelt, *Osmerus eperlanus* and Atlantic salmon, *Salmo salar* because their otoliths are fragile and easily digested. In contrast, sprat, *Sprattus sprattus,* dab, *Limanda limanda* and cod, *Gadus morhua* vertebrae were digested to a considerably higher degree than the otoliths from the same prey species in twenty-two pellets of the cormorant on the Swedish west coast (Härkönen, 1986). Fish have two sagittal otoliths and 40 to 60 vertebrae so the expected ratio between otoliths and vertebrae in the pellets is 1:20 to 1:30, presuming the digestion rate of these structures occurs at an equal rate. The vertebrae were found to be digested to a considerably higher degree than the otoliths. Fish have only two sagittal otoliths but 40 to 60 vertebrae, so otoliths can be used to identify each individual fish and to quantify prey consumption whereas, the vertebrae can be used to identify species presence but not to accurately determine the numbers of prey consumed (Wise, 1980).

Pierce and Boyle (1991) documented a number of limitations in the use of otoliths for identifying fish species consumed by piscivorous marine mammals. Otoliths are usually identifiable to family level but at species level discrimination can be difficult. This is particularly the case between otoliths of the gadids, saithe, *Pollachius virens,* pollack, *Pollachius pollachius* and haddock, *Melanogrammus aeglefinus*, between otoliths of the

pleuronectids, flounder, *Platichthys flesus,* plaice, *Pleuronectes platessa* and dab and between otoliths of the clupeids, sprat and herring. Related species of juvenile fish are particularly difficult to identify because their otoliths are smaller than in large conspecifics (Härkönen, 1986). Some differentiation of the otolith occurs during fish growth and these changes must be considered or misidentification could result. In general, otoliths from juvenile fish are more rounded and features such as the rostrum and antirostrum are weakly developed or lacking. Additional identification problems may arise from the degree of exposure to digestion in the stomach. Rae (1968) suggested that seals may not eat the heads of large fish and this view was corroborated by eyewitness accounts from fishermen. Large fish would therefore be under-estimated in the diet unless other skeletal parts are used to identify their presence. Hall *et al* (1998) found that despite reports of seals removing the heads of large prey items around fishing nets, that large otoliths are recovered from faecal samples providing evidence that seals do consume the heads of large prey.

Otoliths are composed of aragonitic calcium carbonate which is digested when exposed to the gastric acid of the predator digestive system (Murie and Lavigne, 1985). Complete digestion of otoliths from certain species can lead to the under-estimation of the number of prey consumed and the inaccuracy of the relative importance of prey species in the diet (Jobling and Breiby, 1986). In captive feeding trials the recovery rate for otoliths from 1209 experimental fish representing seven key North Sea species ranged from 0% to 89% with a mean of 42% (Tollit, 1996; Tollit *et al,* 1997b). The results were inconsistent however, due to intra- and inter-specific variation between otoliths, differences between experimental conditions and individual seals.

2.1.2. Digestion rate of skeletal remains in seal faeces and cormorant pellets.

Digestion rate greatly affects erosion of otoliths, with the initial digestive stage being more acidic and hence most erosive. Gastric acid with a pH less than 3.5 is needed to erode otoliths together with a long exposure time (Zijlstra and Vaneerden, 1995). pH values were higher in stomachs with a greater food content. The time during which otoliths are held in the stomach and, to a lesser degree, in the intestines influences erosion rate (Härkönen,

1986). Pinnipeds have one of the largest intestines to body length ratios in the animal kingdom despite most animals with long intestines being herbivores. Krockenberger and Bryden (1994) suggest the long intestine of pinnipeds is an adaptation for diving, increasing the functional volume of the digestive tract for more rapid digestion and possibly serving as an extended food storage compartment so digestion can continue in the brief periods when the seal is at the surface. The digestion rate in pinnipeds is particularly rapid, with an average rate of passage of digesta of 5 hours or less. Helm (1983) suggested that in addition the rate of passage of digesta in pinnipeds is increased by their high metabolic rate and the high water content of their digesta. Published rate of passage of digesta in harbour seals ranged from 2.5 to 6.25 hours (Helm, 1984; Markussen, 1993).

The rate of passage of digesta may not however, be an adequate measure of otolith excretion since different parts of food are excreted at different rates (Markussen, 1993). Experiments on four captive harbour seals indicated that the fluids and soft parts of herring were transported more rapidly through the digestive system than the hard parts, although since the soft parts have the higher calorific value, this is inconsistent with the fact that fat delays digestion. Whiting, *Merlangius merlangus* the species with the lowest calorific content, had a faster transit time through the digestive system compared to herring. Transit time of soft parts through the digestive system therefore appeared to be dependent on caloric content of food and on the prey species consumed.

Digestion rate of otoliths varies depending on otolith size, shape and composition (Jobling, 1987). The degree of species and size specific erosion has been widely studied (Da Silva and Neilson, 1985; Jobling, 1987; Prime and Hammond, 1987, Harvey, 1989 and Cottrell *et al*, 1996; Bowen, 2000). Otoliths with high area/volume ratios (small, flat and irregularly shaped otoliths) are more likely to be completely digested than otoliths with low area/volume ratios (Prime and Hammond, 1985). Fishes such as clupeids, osmerids and salmonids, have small and/or fragile otoliths which are vulnerable to degradation or loss in the digestive system whereas gadid otoliths are larger and are among the most resistant (Pierce *et al*, 1993). Jobling and Breiby (1986) compared the digestion rates of herring

(clupeid) and haddock (gadid) otoliths in acidic solution. Herring otoliths were eroded and dissolved more rapidly. The recovery rate for smaller otoliths within and between a prey species may therefore be lower than for the larger otoliths and the numerical importance of small fish is likely to be underestimated. Da Silva and Neilson (1985) suggest that herring otoliths are emptied from seals' stomachs after only a brief time period. In contrast to previous studies, Arim and Naya (2003) predicted that small prey had smaller biases than large prey using a mathematical model.

Captive trials on digestion rates in cormorants found that of thirty pellets regurgitated twenty contained otoliths, four contained other remains and six contained only mucus (Zijlstra and Vaneerden, 1995). In the six pellets containing only mucus, the fish had been completely digested, including bones and scales. The recovery rate of otoliths ingested by cormorants was about 52%, with the majority being found in pellets and only one otolith occurring in the faeces. Otoliths from larger fish had a higher recovery rate with 56% of ruffe, *Gymnocephalus cernua* otoliths consumed recovered compared to only 3% of the perch, *Perca fluviatilis* consumed and none of the bream, *Abramis brama,* roach, *Rutilus rutilus* or pikeperch, *Stizostedion lucioperca* that were consumed. In the field, however, diet research on pellet analysis conducted throughout the year roughly coincided with the birds' theoretical energy demands.

2.1.3. Predicting prey length and body mass from otolith size

Otoliths grow in synchrony with the cranium and so in most species absolute size of otoliths can be correlated to fish length. Fish mass is more variable than fish length depending on time of year, geographic location, sex, reproductive status or fullness of stomach (Frost and Lowry, 1981). Variation in mass can be pronounced in sexually mature individuals at certain times of the year. Linear regression equations from otolith size were found to accurately predict fish length (Härkönen, 1986; Tollit, 1996; Leopold *et al*, 2001). Härkönen (1996) stated that the power function was the best formula for calculating fish mass from fish length or otolith size, since in about 90% of tested species it returned the highest determination coefficient. Tollit (1996) found a two stage regression calculation to

be more applicable for estimating the mass of fish consumed because of seasonal variability of mass. That is to calculate fish length from otolith size using the best-fit regression equation and then calculate fish mass from fish length using the power equation (Coull *et al*, 1989).

Four morphometric parameters of the otolith, length, width, thickness and mass, can be used to estimate fish size (Neilson and Johnson, 1992). It is important that the parameters chosen achieve the highest possible correlation between otolith size and fish size. Otolith length is the largest of the one-dimensional parameters. This minimizes the error of measurement and it is commonly referred to in the literature (Härkönen, 1986; Tollit, 1996, Leopold *et al*, 2001). Pointed otolith tips are easily broken; however; and in some species, such as whiting, herring, sprat, viviparous blenny, *Zoarces viviparous* and butterfish, *Pholis gunnellus* are irregularly shaped, potentially lowering the correlation coefficient. Variability in the ratio of fish length to otolith length and the lengths of left and right otoliths of individual fish are potential sources of error, although otolith length tended to be less variable than otolith width when plotted against fish length (Frost and Lowry, 1981). Prime and Hammond (1987) considered that the reduction in otolith length and width will not be at a constant absolute rate due to different rates of digestion and excretion and so otolith thickness gave a more accurate representation. Härkönen (1986) stated that there will be variability in predicting fish mass from otolith thickness as the measurement could be taken from different areas of the otolith and because they are small, hence vulnerable to relatively large errors. In addition, they are fragile and easily broken during measurement. There is an increased chance of error due to thickness being the smallest measurement (Casteel, 1976). The error of measuring an irregular shape is reduced by measuring otolith mass but error can occur depending on the quality of the balance used. The otoliths must be carefully cleaned using biotex and oven dried to constant weight in order to achieve a similar water content. In addition, mass is a three dimensional parameter and so will be reduced by an exponent of three if eroded.

Different degradation of otoliths affects the accuracy of estimating fish size from otoliths. A mean length reduction of 27.5% (range from 16 to 51%) was found for 1209 experimental fish representing seven key North Sea species (Tollit, 1996; Tollit *et al*, 1997b). Although, small otoliths are more likely to be completely digested than larger otoliths, the mean proportion of size reduction from erosion increased with otolith size and robustness (Tollit, 1996). Length reduction and recovery rate of otoliths were positively correlated with mean otolith length, width and robustness. This is probably a combination of different retention times in the gut, with larger particles tending to be held longer, and the ability to resist digestion. Fish size estimated from otoliths is therefore more likely to be underestimated for larger prey.

2.1.4. The application of correction factors (CF)

A number of experiments on captive seals have attempted to correct for complete digestion from recovery rates and to correct for partial digestion from the reduction in otolith size passing through the digestive system by using correction factors (CFs). The application of increasingly complex species-, size- and grade-specific correction factors, determined from *in vitro* degradation together with data from captive seals, has progressively improved reconstructed estimates of the biomass of prey fed to the seals (Tollit *et al*, 1997b; Bowen, 2000).

The number of species consumed and the size of prey can still be inaccurate however, despite application of CFs. The error in using CFs to correct for erosion of otoliths depends on the model used. Experiments by Tollit (1996) found that the mean mass of fish was underestimated by an average of 48% (ranging from 16-69%) when no CF was applied to correct for erosion of the otoliths, whereas, a species-specific CF applied to correct for the affect of complete digestion on recovery rates and to affect of partial digestion on the reduction in otolith size caused an overestimation by 17% for most species, and a slight under-estimation for sprat, plaice and the larger size ranges of lesser sandeel, *Ammodytes tobianus*, cod and lemon sole, *Microstomus kitt* (Tollit, 1996). The mean mass of the

smallest size range of whiting and lesser sandeel was over-estimated by 69% and 54%, respectively.

The results of captive studies may not reflect the pattern of digestion in the wild as seals are often fed three equal low density meals a day, whilst in the wild they would undergo periods of fasting followed by large meals (Helm, 1983). Pigs fed continually or on an *ad libitum* basis were found to pass their meal faster than animals fed less frequently, suggesting that digestion rates of captive seals are likely to be slower. In addition, higher activity levels of wild seals may be associated with increased movement of digesta (Cottrell *et al*, 1996). Reduced activity tends to cause a reduction in the rate of gastric emptying (Marcus *et al*, 1998) and so a longer time for the complete digestion of otoliths. Reduction in otolith length may also vary with meal size. Herring otoliths recovered from faecal samples of one captive harbour seal pup and eight captive grey seal pups were more eroded after large meals than after half-ration meals (Marcus *et al*, 1998). This is inconsistent with recovery rate and it was suggested that this discrepancy may be explained due to the few herring otoliths surviving the digestion of half-ration meals passing quickly through the stomach and being minimally eroded. Cod otoliths from half-ration meals were more significantly eroded than those from large meals, as expected. Feeding experiments also indicated considerable variation in recovery rate between individual seals (Marcus *et al*, 1998). The age of the seal tends to influence digestion with a faster digestion rate in young seals compared to adults and sex may also influence digestion rate (Krockenberger and Bryden, 1994). Brown and Pierce (1997) found correction factors from captive feeding experiments over-estimated fish size in the diet of harbour seals at Mousa, Shetland due to different digestion rates between those in the stomachs of wild seals compared to those of seals exposed to the artificial conditions of the captive environment and feeding regimes. Brown and Pierce (1998) suggested that inclusion of other fish remains, in addition to otoliths, gave a more representative assessment of seal diet than the using experimentally derived correction factors.

Captive feeding trials on digestion rates in cormorants overestimated digestion rates of skeletal parts due to induced stress in the captive environment (Zijlstra and Vaneerden, 1995). Stress increases calcium secretion in birds, and this increases calcium demand therefore causing increased otolith erosion. Stress may also increase metabolism so further increasing otolith digestion. Calcium demands in birds vary seasonally, such as during egg laying in the female, and otolith digestion depends on calcium demands so the digestion process in cormorants should not be regarded as constant. Zijlstra and Vaneerden (1995) conclude that data from captive trials is unfit for calibration of otolith erosion in dietary studies.

2.1.5. Secondary consumption in seal and cormorant diet

Some researchers have regarded the presence of invertebrate and small fish remains in seal faecal samples (McConnell *et al*, 1984; Prime and Hammond, 1987) and cormorant pellets (Blackwell and Sinclair, 1995) to be a result of secondary consumption. That is the remains were in the digestive system of larger prey rather than consumed by the predators. In cod of up to 50 cm in length, for example, Crustacea often account for at least 30% of the estimated weight consumed (Daan, 1983). Secondary ingestion is a source of error in analysing prey consumption by top predators as it causes an overestimation of invertebrates and small fish in the diet.

Blackwell and Sinclair (1995) reported evidence of secondary consumption in 742 regurgitated pellets of nestling double-crested cormorants, *Phalacrocorax auritus* from ten colonies in Maine, USA. These authors compared lengths of otoliths for a given taxon represented in both pellets and fish stomachs and found overlap in size of prey taken by the cormorants and predatory fish. They concluded that the use of otoliths in diet analysis for double-crested cormorants can inflate estimates of species percent occurrence and number due to secondary consumption and should be used with caution. McConnell *et al* (1984) assumed that Crustacea and polychaete remains in seal faeces were due to secondary ingestion and they were not included in determining prey consumption. Prime and Hammond (1987) considered lesser sandeels present in large quantities to have been

ingested directly but that small numbers present with larger species, particularly gadids, to be the result of secondary ingestion. In a study of seal diet in Scotland, Cottrell *et al* (1996) excluded 30 sandeel otoliths for each large gadid otolith (or large bone) per sample, 20 sandeels for each medium gadid otolith, such as whiting and 10 sandeels per predatory flatfish, to correct for the presumed secondary ingestion of lesser sandeels.

Prime and Hammond (1987; 1990) suggested that Crustacea are a major component of the diet of young seals learning to feed independently and Crustacea remains correlate with period after weaning. The research by Prime and Hammond (1987; 1990) indicates that there is an ontogenetic shift in the diet of harbour seals due to foraging ability with yearlings predominately consuming relatively slow-moving, benthic Crustacea that they can catch on the seabed and then a shift to a predominantly piscivorous diet by the end of the first year as the seals become more efficient at catching fish. A predominately piscivorous diet will provide the seals with a higher energy content than the consumption of small benthic Crustacea.

2.2. METHODOLOGY FOR COLLECTION AND IDENTIFICATION OF OTOLITHS

2.2.1. Collection of harbour seal faecal samples

One hundred and seventy-five visits were made to the mudflats at Greatham Creek, Seal Sands, a haul-out site for harbour seals, between June 1999 and June 2003 (Appendix C). Daily observations of seal summer haul out behaviour, 1989-2003 showed that Greatham Creek was the only seal haul-out site that was used solely by harbour seals and not grey seals (Turner, 2003). Harbour seals are the predominant seal species of interest because they are the breeding residents on the Tees Estuary. Few seal faeces were found per visit in relation to the number of seals present and therefore fortnightly visits on a neap tide were made to provide a large sample size to account for variable foraging due to season, sex, age and individuals. Faecal samples were only obtained during 63 of these visits. More faecal samples would not have been obtained if more regular visits had been made since samples would be washed away on a higher tide. Visits were conducted at low tide as the seals were known to swim downstream to haul out on the Seal Sands mudflats before low tide. The seals were therefore not disturbed. Foot and Mouth Disease restrictions prevented visits to the site during the summer of 2001 so seal faecal samples could not be collected.

Each faecal sample was scooped up using a knife with a flat blade and placed into a large, plastic container. The containers were labelled with date and collection site and frozen at -20°C.

2.2.2. Collection of cormorant pellets

Twenty cormorant pellets were collected every two months between January 2000 and December 2002 from the last two structures of Phillips Jetty, Seal Sands. Herring gulls, *Larus argentatus* also roosted on the jetty and produce pellets but during weekly WeBs bird counts made since 1992 they were only observed on the structures closest to shore and were not observed on the end two structures of the Jetty (R. Ward, Durham University, pers. comm.). It was therefore assumed that all regurgitate samples came from cormorants. The

pellets were collected using latex gloves and put into individual plastic pots. These pots were later labelled with date and collection site and frozen at -20°C.

2.2.3. Processing of faecal samples and pellets

Faecal samples and pellets were examined within two weeks of freezing because the hard parts can degrade due to elapsed time between defecation and collection, freezing and processing, the acidity of the sample (related to the amount of food in the gut prior to defecation) and differences in the ambient temperature (Prime and Hammond, 1987). The material was defrosted and washed through a nest of three brass sieves of decreasing mesh size of 2 mm, 0.5 mm and 0.25 mm to separate the hard parts from the rest of the faecal sample. The hard parts were retained by the sieves and extracted using fine point forceps, whereas the remainder of the faecal sample was washed through the sieve and discarded. The hard parts from the seal faecal samples were more easily seen if left to dry under a lamp before extraction, so this method was used. After sorting, otoliths and smaller hard parts were stored dry in individual wells in a plastic tray containing 24 wells. Each sample was stored separately in one to three trays, depending on the number of parts. Larger hard parts and invertebrate parts were stored within screw top plastic pots, to prevent degradation. The invertebrate parts were stored in 70% ethanol. All containers were labeled with date, collection site, whether from cormorant pellets or seal faeces and numbered so it was known which hard parts came from individual samples. Records were made of each hard part extracted and referenced to the container with date, predator species, collection site, sample number, species and size measurements.

The length and width of otoliths were measured using a binocular dissecting microscope and digital callipers, to a precision of 0.01 mm. Most otoliths were measured except in cases where small otoliths of ammodytid, *Trisopterus* or pleuronectid otoliths occurred in large quantities. In these cases, the otoliths were counted and sub-samples were measured.

2.2.4. Species identification of prey remains

Invertebrate remains were identified using a reference collection of Crustacea from the Hartlepool Power Station intake water, a collection of identified mollusc shells, identification training by an expert (T. Mercer, Aquatic Environments) and a reference guide (Hayward and Ryland, 1996). The presence of invertebrate remains in each faecal sample and pellet was compared with the presence of the main fish species to determine whether consumption was likely to be due to secondary consumption or whether invertebrates are directly consumed by seals and cormorants. Crustacea were identified from their exoskeleton fragments and chelae, molluscs were identified from their shell fragments and polychaetes were identified from their jaws. The number of crabs consumed was obtained from the number of chelae divided by two and rounded to the lowest integer. Fragments of exoskeleton were assumed to have come from the same crab if chelae were present or if chelae were absent this was counted as one crab. The number of common shrimp was counted from the presence of their exoskeleton. The number of molluscs consumed was obtained from the number of shells present and for *M.edulis* each half of shell counted was divided by two and rounded to the lowest integer. The number of polychaetes consumed was identified from the number of jaws divided by two and rounded to the lowest integer.

The species of fish consumed by each predator were identified from otoliths and bones. These hard parts were compared with a reference collection, comprising otoliths and bones of fish collected from Hartlepool Power Station intake water, a reference collection of fish otoliths from the North Sea collected by M. Lucas, otolith reference guides (Härkönen, 1986; Leopold *et al*, 2001) and fish bone reference guides (Watt *et al*, 1997). A binocular dissecting microscope was used to identify the features of smaller hard parts. The otoliths of 0-group pleuronectids could not be identified to species because they were so small that they could not be distinguished. These otoliths were classified to family.

Pierce *et al* (1990) stated that identification and frequency of most fish species in faecal samples can be markedly improved by the use of other skeletal remains, in addition to

otoliths. Other skeletal bones were therefore also collected and used as an additional identification tool for several fish species. The characteristic fish bones that could be easily used for identification were dependent on the family: Clupeidae (otic bulla, vertebrae and premaxilla); Gadidae (pre-maxillae, maxillae, vertebrae and prevomer); Pleuronectidae (pre-maxillae, maxillae, vertebrae and urohyals); Callionymidae (vertebrae and pre-opercular spines); Carangidae (vertebrae and scutes); trachinidae (spines), Cyprinidae and Labridae (pharyngeal teeth) and vertebrae for all other species.

2.3. METHODS FOR IDENTIFICATION OF PREY REMAINS AND ESTIMATION OF PREY SIZE

Fresh specimens of 475 fish representing ten species were collected from the Hartlepool Power Station cooling water intake bi-monthly between June 1999 and December 2002. The number of each species collected bi-monthly and the length and mass were recorded (Table 2.1). Total length was measured from the tip of the snout to the tip of the caudal fin to the nearest 0.1 mm with a digital calliper. Standard length was measured from the tip of the snout to the base of the fish body, before the caudal fin, to the nearest 0.1 mm with a digital calliper. The fish were weighed wet to the nearest 0.01 g.

Table 2.1. The sample size of each species and the range of total body lengths (mm) and body mass (g) of prey fish collected from the Hartlepool Power Station intake water, June 1999 and December 2002

Species	*n*	Total length (mm)	Mass (g)
Whiting	115	86 - 342	5.43 - 216.04
Cod	46	67 - 285	2.16 - 279.81
Saithe	37	67 - 260	2.77 - 80.39
Shore rockling	8	132 - 205	14.63 - 74.40
Sprat	43	85 - 142	2.62 - 24.41
Herring	39	84 - 264	3.22 - 86.57
Flounder	83	97 - 345	9.21 - 535.05
Plaice	11	53 - 210	1.62 - 96.73
Lesser sandeel	41	146 - 190	10.75 - 24.02
Lesser weever	48	86 - 149	5.21 - 31.43

Otoliths were dissected from the fish using a sharp knife to open the skull in the position of the labyrinths and fine forceps to carefully remove the otoliths. To obtain other bones the fish were microwaved for 2 to 8 minutes to remove the bulk of the flesh. The bones were then placed in a detergent mix (Biotex) to clean them of excess flesh. The length and width of otoliths and bones were measured using a binocular dissecting microscope with graticule or with a caliper. Otoliths with pointed tips may break easily and some are irregular shapes. Otoliths with broken tips were discarded. Only the author conducted the measurements to gain consistency and precision in measuring otolith size from these known size individuals.

2.3.1. Statistical analysis

Kolmogorov-Smirnov tests were conducted to assess whether the fish size data differed significantly from a normal distribution. The data was not significantly different to normal for all species and parametric statistics were used. Least squares regression equations were generated using SPSS to predict original total fish length from measurements of otolith length and otolith width. The use of otolith length or otolith width as the independent variable was compared to identify the best independent variable for predicting fish length in known size fish (Appendix Di and Diii).

Regression equations from known size fish from the Tees Estuary therefore could not be used to predict the size of fish consumed by seals or cormorants for any fish species, except sprat because the range of otolith lengths and widths collected from these known size fish were smaller than the range of otolith sizes extracted from seal faeces and cormorant pellets for all fish species, except sprat. In addition, only ten species of fish from the Tees Estuary were measured, whereas a total of 29 otoliths were found from different fish species in excreted matter. The otolith sizes were within the same range for known size sprat and those extracted from seal faeces and cormorant pellets and lengths of known size sprat were less than the lower values of the 95% prediction interval when predicted using published linear regression equations, so sprat length was predicted using regression coefficients predicted from known size sprat from the Tees Estuary. Regression equations from other published studies were generated for fish of known body length and mass (Härkönen, 1986;

Tollit, 1996, Leopold *et al*, 2001) and the strength of the regression equations were compared to identify the best variable for predicting fish length and mass (Appendix Dii and Div). Linear regression equations published in Leopold *et al* (2001) estimated values closest to the known size fish lengths for all species, except sprat and also five-bearded rockling, wrasse and unidentified pleuronectids since Leopold *et al* (2001) did not provide linear regression equations for the latter three types. Regression equations using otolith length as the independent variable published by Härkönen (1986) were used for five-bearded rockling, wrasse and unidentified pleuronectids. All equations for calculating fish length published by Leopold *et al* (2001) had to be multiplied by 10 so length was expressed in mm rather than cm.

The power function was found to be the most accurate method of estimating fish body mass. The independent variable that predicted fish mass the most accurately was otolith length rather than fish length, for all species (Appendix Diii). The reliability of each predicted fish size was described using upper and lower values of the 95% prediction interval. Power regression equations published in Leopold *et al* (2001) estimated values closest to the known size fish mass for all species from the Tees Estuary, except sprat and also five-bearded rockling, wrasse and unidentified pleuronectids (Appendix D iv). Body mass of known size sprat were less than the lower values of the 95% prediction interval when predicted using published linear regression equations. Sprat mass was therefore predicted using power regression coefficients predicted from known size sprat from the Tees Estuary. There were no power equations for five-bearded rockling, wrasse and unidentified pleuronectids published by Leopold *et al* (2001) so equations published by Härkönen (1986) using otolith length as the independent variable were used.

2.4. RESULTS OF IDENTIFICATION OF PREY REMAINS AND ESTIMATION OF PREY SIZE CONSUMED BY HARBOUR SEALS AND CORMORANTS

2.4.1. Prey species identified in harbour seal diet

A total of 7 families and 15 species of fish were identified. Table 2.2 lists the fish species consumed. The total and seasonal abundance of each fish species in the seal diet is shown in Chapter 3.

Table 2.2. Families and species of fish identified from faecal samples collected from the Tees Estuary, 1999 - 2003

FISH Family	Species Name	Common Name
Clupeidae	*Clupea harengus*	Herring
	Sprattus sprattus	Sprat
Gadidae	*Ciliata mustela*	Five-bearded rockling
	Gadus morhua	Cod
	Melanogrammus aeglefinus	Haddock
	Merlangius merlangus	Whiting
	Pollachius virens	Saithe
	Trisopterus minutus	Poor cod
Trachinidae	*Echiichthys vipera*	Lesser weever
Zoarcidae	*Zoarces viviparus*	Eelpout
Ammodytidae	*Ammodytes tobianus*	Lesser sandeel
Callionymidae	*Callionymus lyra*	Common dragonet
Pleuronectidae	*Limanda limanda*	Dab
	Platichthys flesus	Flounder
	Pleuronectes platessa	Plaice

A total of 8 families and 7 species of macroinvertebrates were identified. Table 2.3 lists the invertebrate species consumed. The total and seasonal abundance of invertebrate species in the seal diet is shown in Chapter 3.

Table 2.3. Invertebrates consumed by harbour seals from the Tees Estuary, June 1999 - June 2003

Phylum	Class	Order	Family	Species name	Common name
Crustacea	Malacostraca	Decapoda - Reptantia	Portunicidae	*Carcinus maenas*	Shore crab
Crustacea	Malacostraca	Decapoda - Reptantia	Portunicidae	*Liocarcinus depurator*	Swimming crab
Crustacea	Malacostraca	Decapoda Natantia	Crangonidae	*Crangon crangon*	Common shrimp
Mollusca	Bivalvia		Mytilidae	*Mytilus edulis*	Common mussel
Mollusca	Bivalvia		Scrobiculariidae		
Mollusca	Bivalvia		Tellinidae		
Mollusca	Gastropoda	Mesogastropoda	Littorinidae	*Littorina littorea*	Common periwinkle
Mollusca	Gastropoda	Mesogastropoda	Hydrobiidae	*Hydrobia ulvae*	Laver spire shell
Annelida	Polychaeta		Nereidae	*Neanthes virens*	King ragworm

2.4.2. Prey species identified in cormorant diet

A total of 28 species of bony fish (Osteichthyes) comprising 17 family groups were identified from otoliths and other bones (Table 2.4). The total and seasonal abundance of each fish species in the cormorant diet is shown in Chapter 3.

Table 2.4. Fish species present in cormorant pellets collected from Seal Sands, January 2000-December 2002

Family	Species Name	Common Name
Clupeidae	*Clupea harengus*	Herring
	Sprattus sprattus	Sprat
Gadidae	*Ciliata mustela*	5 bearded rockling
	Gadus morhua	Cod
	Melanogrammus aeglefinus	Haddock
	Merlangius merlangus	Whiting
	Pollachius virens	Saithe
	Trisopterus minutus	Poor cod
Triglidae	*Eutrigla gurnardus*	Grey gurnard
Cottidae	*Myoxocephalus scorpius*	Bullrout
	Taurulus bubalis	Long-spined sea scorpion
Carangidae	*Trachurus trachurus*	Scad
Labridae	Labridae spp.	Wrasse species
Trachinidae	*Echiichthys vipera*	Lesser weever
Zoarcidae	*Zoarces viviparous*	Eelpout
Pholididae	*Pholis gunnellus*	Butterfish
Ammodytidae	*Ammodytes tobianus*	Lesser sandeel
Callionymidae	*Callionymus lyra*	Dragonet
Gobiidae	*Pomatoschistus minuta*	Sand goby
Scophthalmidae	*Lepidorhombus whiffiagonis*	Megrim
Pleuronectidae	*Hippoglossoides platessoides*	Long rough dab
	Limanda limanda	Dab
	Platichthys flesus	Flounder
	Pleuronectes platessa	Plaice
Soleidae	*Solea solea*	Dover sole
Cyprinidae	*Rutilus rutilus*	Roach
	Cyprinid species	Other unidentified cyprinids
Percidae	*Perca fluviatilis*	Perch

Five hundred and thirty invertebrate remains were found in 146 pellets. Seventeen family groups and twelve species of invertebrates were identified Table 2.5. The total and seasonal abundance of invertebrate species in the cormorant diet is shown in Chapter 3.

Table 2.5. Invertebrate remains in cormorant pellets collected from Seal Sands, January 2000-December 2002

Phylum	Class	Order	Family	Species name	Common name
Crustacea	Malacostraca	Decapoda - Natantia	Crangonidae	*Crangon crangon*	Common shrimp
Crustacea	Malacostraca	Decapoda - Natantia	Palaemonidae		
Crustacea	Malacostraca	Decapoda -Reptantia	Portunicidae	*Carcinus maenas*	Shore crab
Crustacea	Malacostraca	Decapoda - Reptantia	Portunicidae	*Liocarcinus depurator*	Swimming crab
Crustacea	Malacostraca	Decapoda - Reptantia	Cancridae	*Cancer pagarus*	Edible crab
Crustacea	Malacostraca	Decapoda - Reptantia	Paguridae	*Pagurus bernhardus*	Hermit crab
Crustacea	Malacostraca	Decapoda - Reptantia	Galatheidae		
Crustacea	Cirripedia	Thoracica	Balanidae		
Mollusca	Bivalvia		Mytilidae	*Mytilus edulis*	Common mussel
Mollusca	Bivalvia		Scrobiculariidae		
Mollusca	Bivalvia		Tellinidae		
Mollusca	Bivalvia		Donacidae	*Donax vittatus*	Banded wedge shell
Mollusca	Gastropoda	Neogastropoda	Muricidae	*Nucella lapillus*	Dog whelk
Mollusca	Gastropoda	Mesogastropoda	Littorinidae	*Littorina littorea*	Common periwinkle
Mollusca	Gastropoda	Mesogastropoda	Hydrobiidae	*Hydrobia ulvae*	Laver spire shell
Mollusca	Gastropoda	Archaeogastropoda	Trochidae	*Gibbula cineraria*	Grey top shell
Mollusca	Gastropoda	Archaeogastropoda	Patellidae		
Annelida	Polychaeta		Nereidae	*Neanthes virens*	King ragworm

2.5. FISH SIZE PREDICTIONS

Otolith length was used to predict fish length rather than otolith width because although linear regression equations between fish length and otolith length and otolith width both provided significant results, otolith length was the strongest predictor of fish length (Appendix D i). Otoliths with a broken tip were excluded from the analysis.

Sprat length was predicted using regression coefficients predicted from known size sprat from the Tees Estuary ($r^2 = 0.95$, $df = 40$, $p < 0.001$). The length of all other fish species were predicted using the published regression equations that provided the stronger predictor of known size fish. The best fit linear regression equation used to predict fish length for each fish species are given in Appendix Ei.

The body mass of sprat was predicted using power regression coefficients predicted from known size sprat from the Tees Estuary ($r^2 = 0.88$, $df = 40$, $p < 0.001$). There were no power equations for five-bearded rockling, wrasse and unidentified pleuronectids published by Leopold *et al* (2001) so equations published by Härkönen (1986) using otolith length as the independent variable were used. The best fit power regression equations used to predict the mass of fish from otolith length are given in Appendix Eii).

The median and range of fish lengths and mass for each of the main prey species consumed by seals is given below (Table 2.6).

Table 2.6. Median and range of lengths and body mass of fish species consumed by seals from the Tees Estuary, June 1999- June 2002

Species	No. of faeces	No. consumed	Median length (mm)	Range of length (mm)	Median body mass(g)	Range of body mass (g)
Clupeiformes						
Herring	13	21	63	37-304	28.6	0.6-190.1
Sprat	21	66	82	43-148	2.9	1.1-24.2
Gadiformes						
5 bearded rockling	3	6	139	112-153	23.9	11.8-30.4
Cod	39	101	235	30-443	90.1	1.1-730.8
Haddock	1	1	69	69	4.2	4.2
Whiting	45	317	145	51-300	30.3	0.8 – 134.7
Saithe	10	15	163	119-237	33.4	8.1-91.8
Poor cod	24	116	183	95-262	190.4	31.6-537.7
Perciformes						
Lesser weever	2	3	70	68-71	7.9	7.9
Eelpout	3	4	155	142-165	20.5	15.1-25.3
Lesser sandeel	2	12	146	126-172	12.2	10.7-15.2
Dragonet	5	21	187	122-265	2.8	1.0-52.8
Pleuronectiformes						
Dab	1	1	47	47	1.2	1.2
Flounder	15	47	139	46-251	30.1	1.1-180.0
Plaice	8	41	74	47-159	6.3	1.4-39.2
0-group pleuronectids	39	995	54	12-225	4.0	0.5-125.1

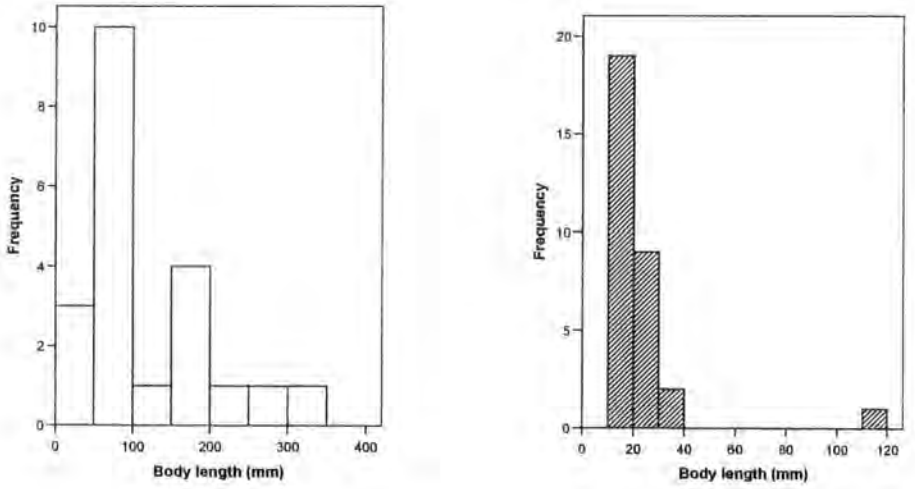
The median and range of lengths and mass for each of the main fish species consumed by cormorants is given below (Table 2.7).

Table 2.7. Median and range of lengths and body mass of fish species consumed by cormorants collected from Seal Sands, January 2000-December 2002

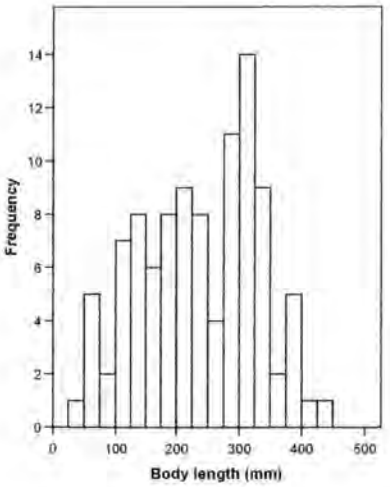
Species	No. of pellets	No. consumed	Median length (mm)	Range of length (mm)	Median body mass (g)	Range of body mass (g)
Clupeiformes						
Herring	17	33	193	100 - 308	32.9	6.7 – 196.4
Sprat	2	2	116	90 – 142	10.9	2.3 – 19.5
Gadiformes						
5 Bearded rockling	11	26	215	211 – 218	106	10.7 – 219.4
Cod	110	436	131	21 – 443	56.5	0.4 – 730.8
Haddock	18	72	161	66 – 266	47.3	2.9 – 175.6
Whiting	139	887	139	31 - 335	13.8	1.0 – 257.2
Saithe	67	204	141	44 - 330	22.9	1.1 – 248.9
Poor cod	17	77	175	52 – 253	65.8	2.1 – 186.5
Scorpaeniformes						
Bullrout	18	40	160	56 – 255	91.3	3.2 – 276.7
Sea scorpion	1	2	180	170 – 190	106.8	87.0 – 126.6
Grey gurnard	2	4	165	120 – 189	38.1	13.3 – 52.9
Perciformes						
Scad	13	35	219	96 – 305	117.4	9.7 – 252.4
Wrasse	2	9	173	68 - 212	25.0	10.1 – 40.5
Lesser weever	90	1408	72	44 – 136	6.2	1.2 – 31.7
Eelpout	3	9	110	105 – 147	6.7	5.6 – 16.9
Butterfish	1	2	166	148 – 183	20.3	12.9 – 27.5
Lesser sandeel	33	388	127	97 – 237	7.1	2.3 – 313.1
Dragonet	48	173	138	63 – 282	22.5	2.4 – 183.1
Pleuronectiformes						
Megrim	1	1	245	245	212.5	212.5
Long rough dab	1	2	235	232 – 239	101.5	96.7 – 106.3
Dab	17	69	174	55 – 351	69.0	1.7 – 585.2
Flounder	83	394	156	36 – 289	49.6	1.8 – 154.3
Plaice	72	318	136	42 - 329	32.2	1.1 – 292.5
0-group pleuronectids	136	1427	77	22 – 260	9.8	0.7 – 157.8
Sole	1	1	194	194	57.4	57.4
Freshwater						
Perch(Perciformes)	17	115	145	57 – 233	39.8	1.8 – 176.4
Roach (Cypriniformes)	21	247	145	97 – 399	46.1	8.4 – 831.8

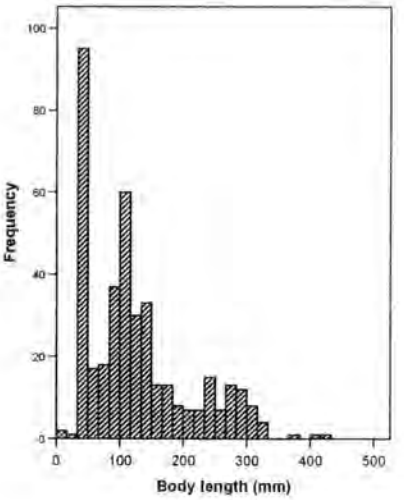
Length frequency distributions were conducted for each of the main fish species consumed by seals and cormorants (Figure 2.3). There was a greater range of body lengths of herring consumed by seals than by cormorants and a similar range of body lengths of cod, whiting, saithe, poor cod, pleuronectids and dragonets consumed by both predators. The length frequency distributions indicated that both predators preferred smaller individual herring and pleuronectids and cormorants preferred smaller cod and whiting. The length frequency distributions for saithe, poor cod and dragonet indicated that both predators prefer medium-sized individuals of these species and the length frequency distributions for cod and whiting indicated that seals tended to take larger cod and whiting than cormorants.

a)

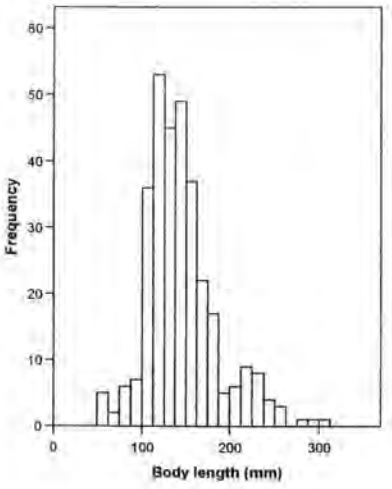


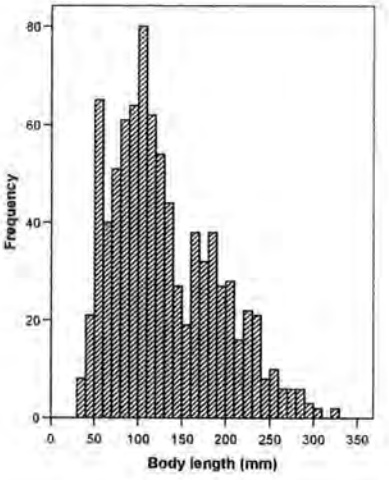
b)


Frequency
Body length (mm)

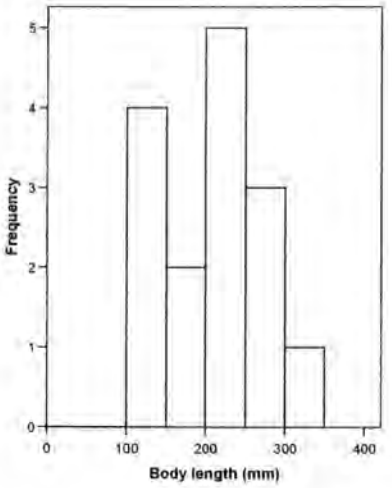

Frequency
Body length (mm)

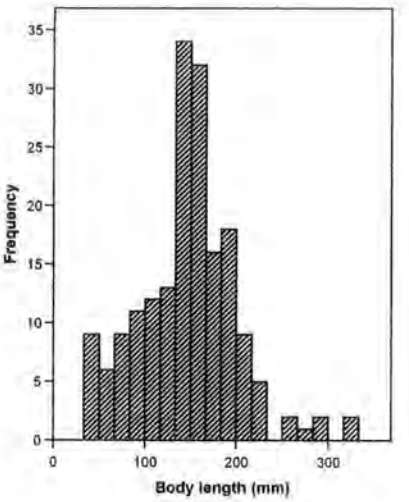
c)


Frequency
Body length (mm)


Frequency
Body length (mm)

d)


Frequency
Body length (mm)
0
1
2
3
4
5
0
100
200
300
400


Frequency
Body length (mm)
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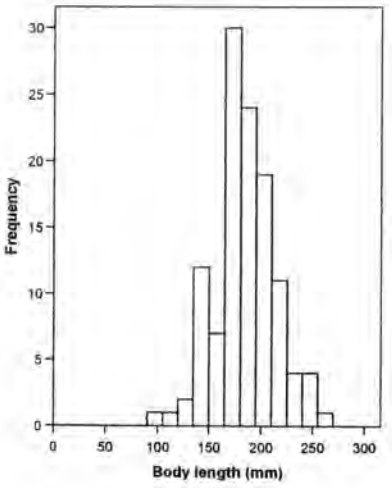
5
10
15
20
25
30
35
0
100
200
300

e)


Frequency
Body length (mm)
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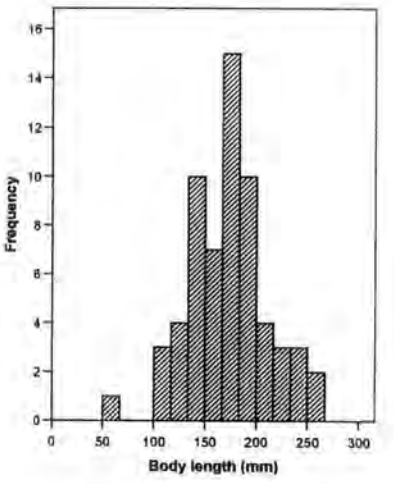
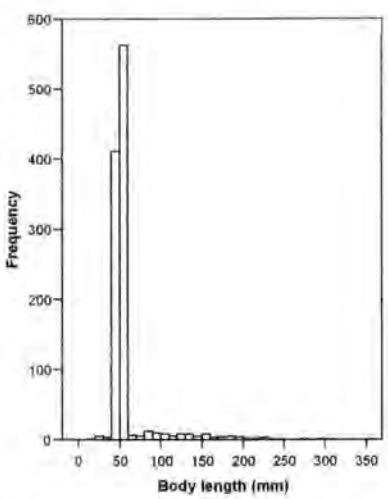
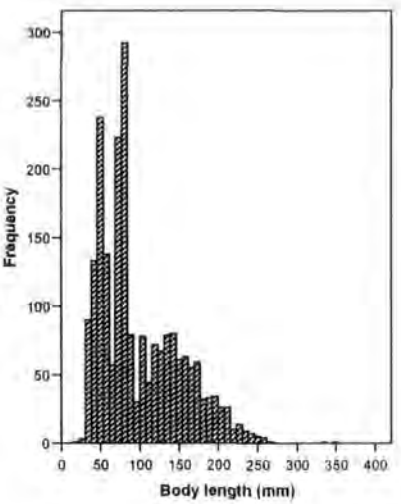
10
15
20
25
30
0
50
100
150
200
250
300

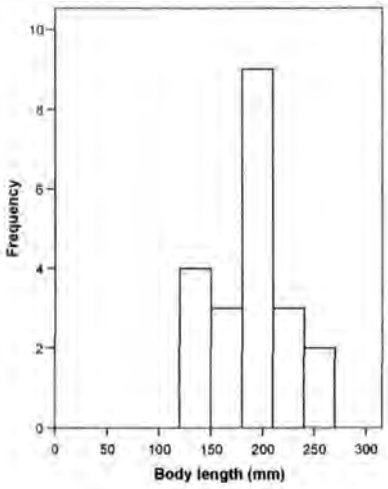

Frequency
Body length (mm)
0
2
4
6
8
10
12
14
16
0
50
100
150
200
250
300

f)





g)



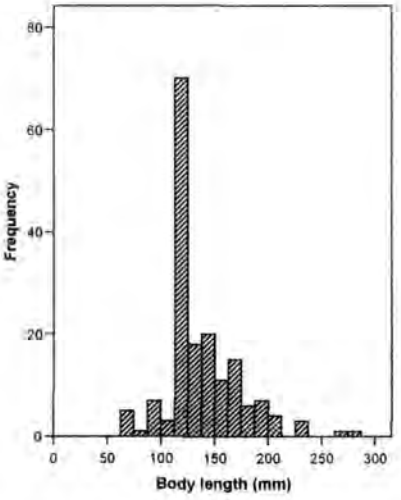


Figure 2.3. Frequency analysis of body length of main fish species consumed by seals (open bars on the left) and cormorants (filled bars on the right) a) herring b) cod c) whiting d) saithe e) poor cod f) pleuronectids g) dragonet

A length frequency distribution was conducted for sprat consumed by seals only, since they are a main prey of seals, whereas few sprat are consumed by cormorants (Figure 2.4). The length frequency distribution indicated that seals from the Tees Estuary predate on medium sized sprat most frequently.

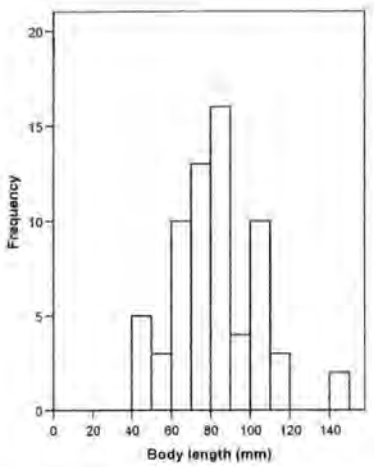


Figure 2.4. Frequency analysis of body length of sprat consumed by seals

Length frequency distributions were conducted for lesser weever, lesser sandeel, perch and roach consumed by cormorants only, since they are a main prey of cormorants but few lesser weever and lesser sandeel are consumed by seals and no freshwater fish are consumed by seals (Figure 2.5). The length frequency distributions indicated that cormorants predated on smaller weever, sandeel and roach most frequently, whereas cormorants preferred medium-sized perch.

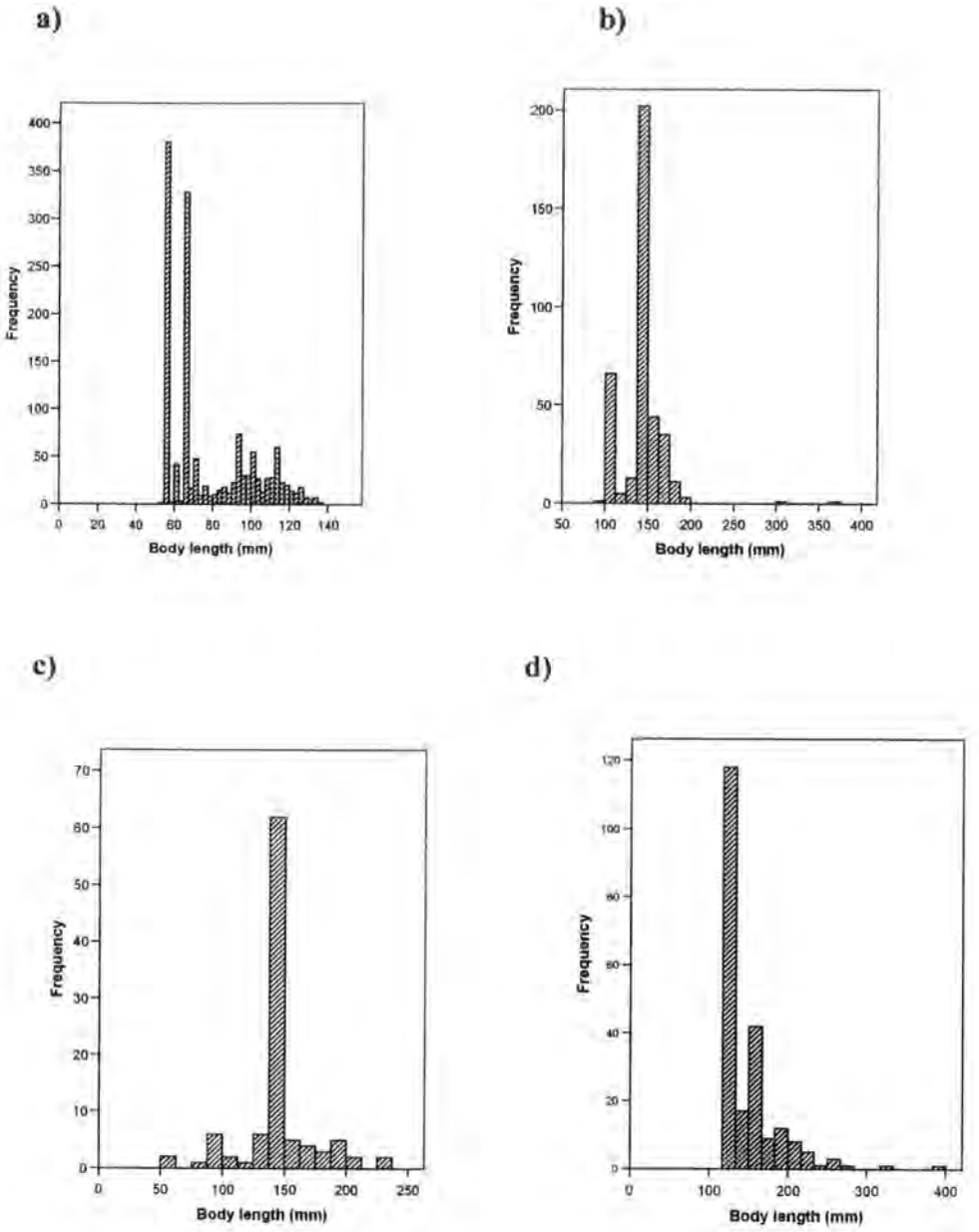
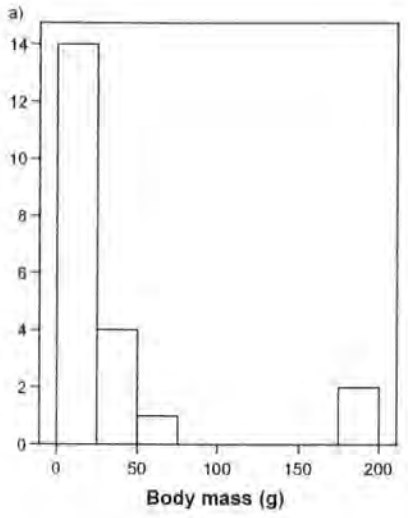
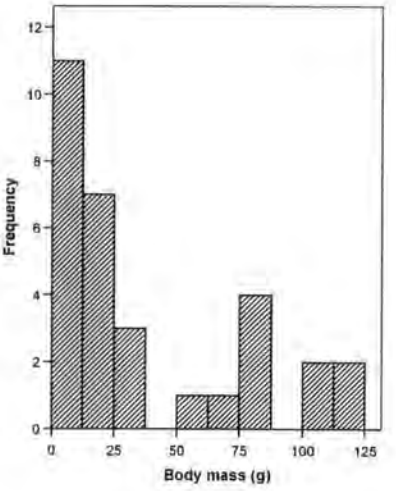


Figure 2.5. Frequency analysis of body length of main fish species consumed by cormorants a) lesser weever b) lesser sandeel c) perch d) roach

Biomass frequency distributions were conducted for each of the main fish species consumed by seals and cormorants (Figure 2.6). The biomass frequency distributions indicate that seals and cormorants predate on smaller individuals most frequently.

a)





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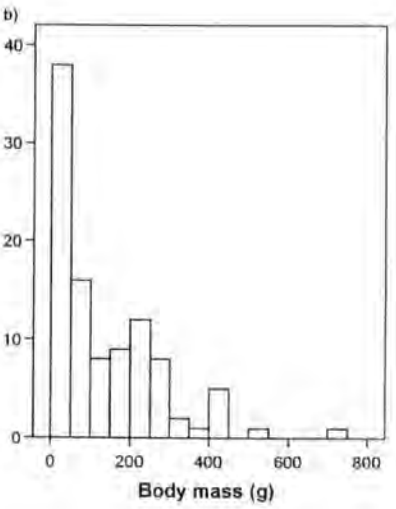
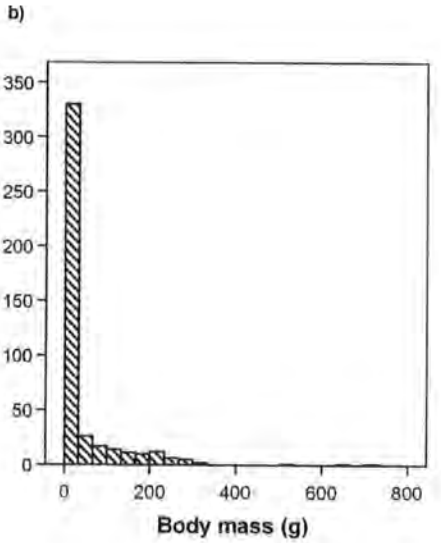
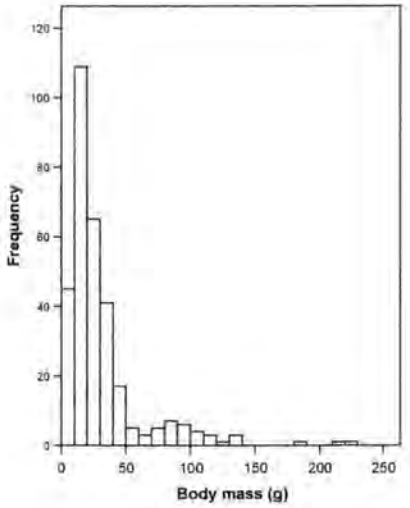
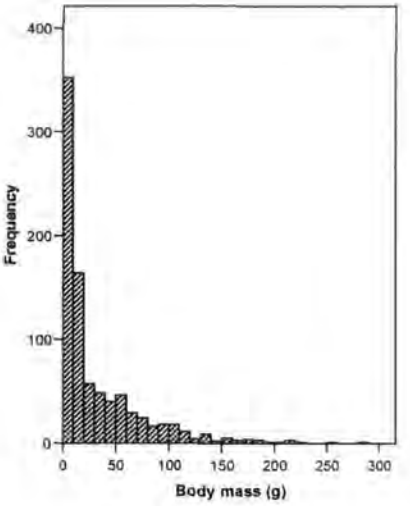




c)


Frequency
Body mass (g)
0
20
40
60
80
100
120
0
50
100
150
200
250


Frequency
Body mass (g)
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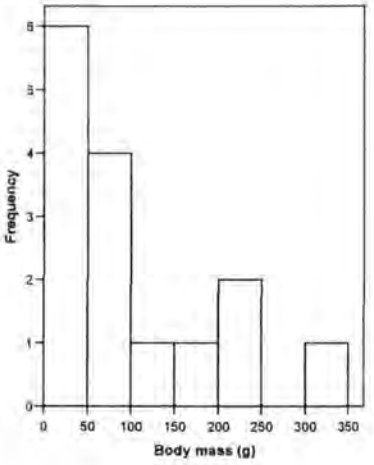
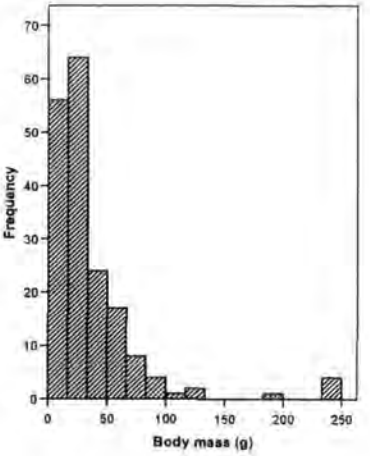
100
200
300
400
0
50
100
150
200
250
300

d)


Frequency
Body mass (g)
0
1
2
3
4
0
50
100
150
200
250
300
350


Frequency
Body mass (g)
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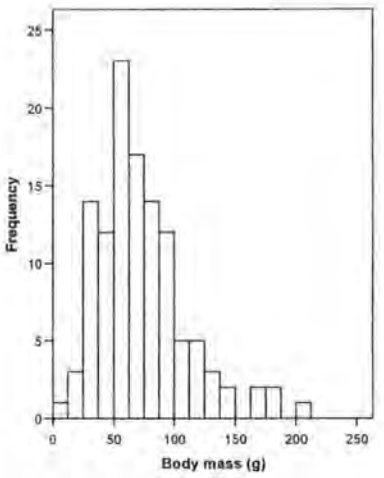
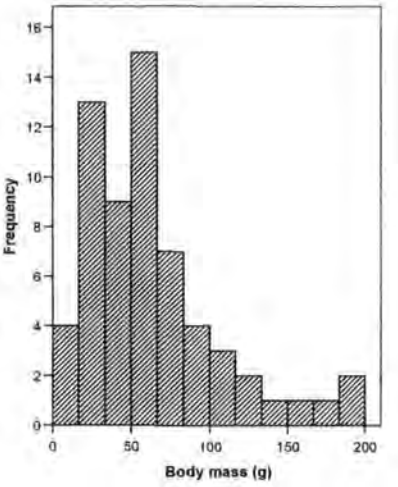
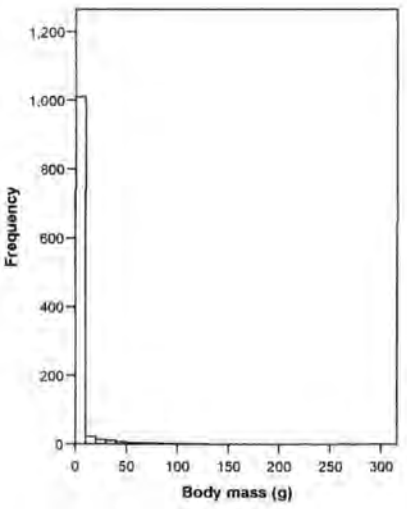
10
20
30
40
50
60
70
0
50
100
150
200
250

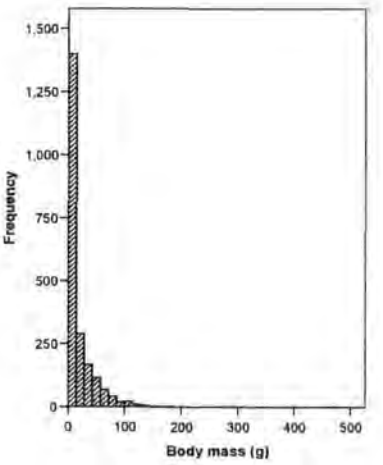
e)


Frequency
Body mass (g)
0
5
10
15
20
25
0
50
100
150
200
250


Frequency
Body mass (g)
0
2
4
6
8
10
12
14
16
0
50
100
150
200

f)


Frequency
Body mass (g)
0
200
400
600
800
1,000
1,200
0
50
100
150
200
250
300


Frequency
Body mass (g)
0
250
500
750
1,000
1,250
1,500
0
100
200
300
400
500

g)

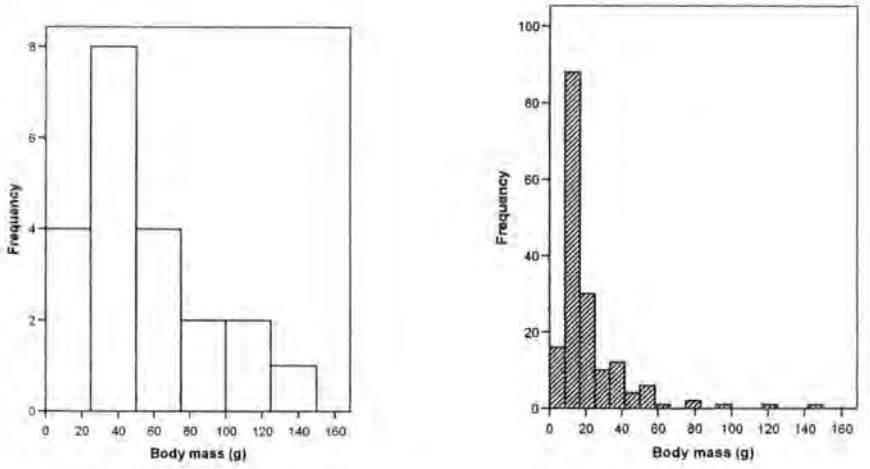


Figure 2.6. Frequency analysis of body mass of main fish species consumed by seals (open bars on the left) and cormorants (filled bars on the right) a) herring b) cod c) whiting d) saithe e) poor cod f) pleuronectids g) dragonet

Biomass frequency distributions were conducted for sprat consumed by seals only, since they are a main prey of seals, whereas few sprat are consumed by cormorants (Figure 2.7). The biomass frequency distribution indicated that seals predate on smaller individuals most frequently.

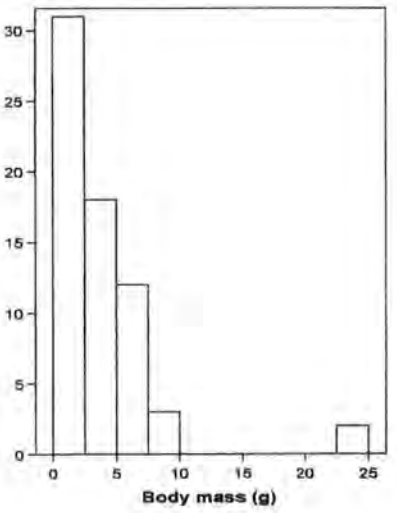
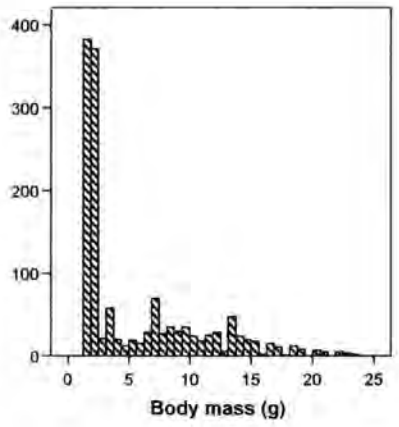


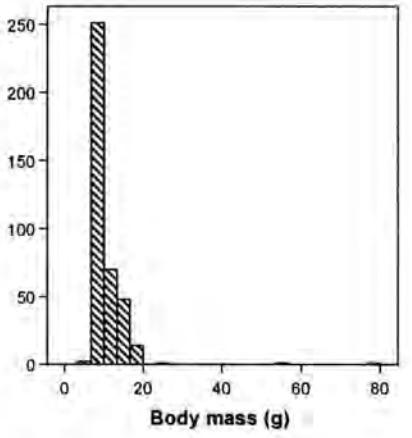
Figure 2.7. Frequency analysis of body mass of sprat consumed by seals

Biomass frequency distributions were conducted for lesser weever, lesser sandeel, perch and roach consumed by cormorants only, since they are a main prey of cormorants but few lesser weever and lesser sandeel are consumed by seals and no freshwater fish are consumed by seals (Figure 2.8). The biomass frequency distributions indicated that cormorants predated on smaller individuals most frequently for each species.

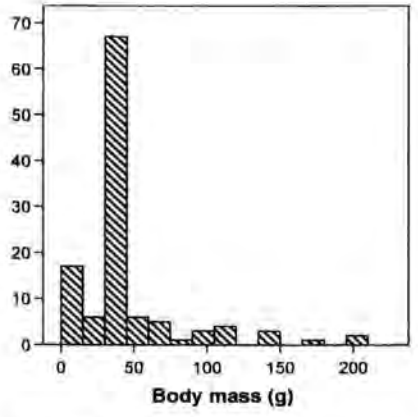
a)



b)



c)



d)

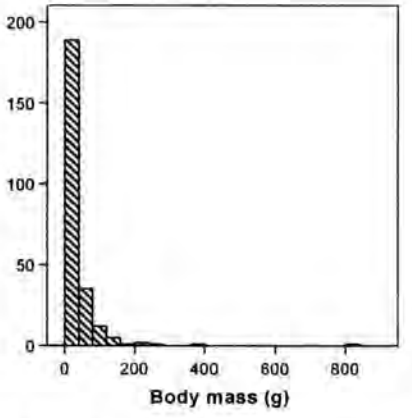


Figure 2.8. Frequency analysis of body mass of main fish species consumed by cormorants a) lesser weever b) lesser sandeel c) perch d) roach

2.6. DISCUSSION OF FISH AND CRUSTACEAN SPECIES CONSUMED BY HARBOUR SEALS AND CORMORANTS

Comparison of the diet of these two opportunistic top predators provides a good indicator of prey availability in the Tees Estuary. There was partial dietary overlap of the prey species consumed by harbour seals and cormorants from the Tees Estuary, although cormorants consumed thirteen fish species not detected in the seal diet, including two freshwater fish species. Prey remains in cormorant pellets are regurgitated rather than passing through the complete digestive system so they are expected to be less eroded by digestive acids than prey remains in seal faeces and may therefore provide a more reliable method of determining the prey species consumed than prey remains recovered from seals faeces. In addition, cormorants void one pellet per day containing all remains of the previous days' meal (Zijlstra and Vaneerden, 1995), whereas harbour seals may void a number of faecal samples a day. Otoliths were the most frequent skeletal parts in both seal and cormorant excretory matter, but other bones were used to corroborate identification and increased the estimated quantity of prey consumed by 3 % in the seal diet and 0.9 % in the cormorant diet. Brown and Pierce (1998) suggested that inclusion of other fish remains, in addition to otoliths, gave a more representative assessment of seal diet than the using experimentally derived correction factors (CFs).

The main disadvantages of using skeletal remains in excretory matter to determine piscivorous diet are otolith digestion rates may vary by species with the potential for small or fragile otoliths, such as those from clupeids and salmonids, being completely digested. More robust otoliths such as those of gadids are less likely to completely digest but they may partially erode leading to an under-estimate in the size of prey consumed. In addition, seals may not consume the heads of large fish, and therefore not the otoliths, of large prey items and some otoliths recovered may be a result of secondary consumption. These factors would potentially bias the numbers of prey species consumed and digestion rates would bias prey size predictions.

Application of CFs for different digestion of species-specific otoliths have been determined to try to produce a more realistic view of the relative number and size of species consumed (Da Silva and Neilson, 1985; Prime and Hammond, 1985; Prime and Hammond, 1987; Harvey, 1989; Cottrell, 1996; Tollit *et al*, 1997b; Marcus *et al*, 1998; Bowen, 2000). CFs however, have limitations. Experiments to determine species-specific CFs were carried out on captive seals, which were not representative of wild seals. There is considerable variation in the recovery rate between individual seals (Marcus *et al*, 1998). Average recovery rates of herring otoliths in faeces of captive harbour seals varied from 11% of the total herring ingested and 4% of herring of 30-35 cm in length from one seal (Da Silva and Neilson, 1985); 33% from six seals (Harvey, 1989), 30% from four seals (Cottrell *et al*, 1996) and 43.7% of herring otoliths from one harbour seal pup and nine grey seal pups (Marcus *et al*, 1998). Recovery of gadid otoliths in faeces varied from 86% from one harbour seal to 73% from six seals (Harvey, 1989) and 81.6% in nine grey seal pups (Marcus *et al*, 1998). The application of CFs will therefore depend on the individual captive seals that it is based upon. The species-specific CF to apply has not been agreed between studies and CFs are expected to vary with region and season to some extent. It is not possible in the field to know the identity of the seals or cormorants voiding the excretory matter in order to control for factors such as the age, sex and activity level or to know the size and frequency of the meal. It was decided not to apply CFs to this study due to these limitations. The data in this chapter are minimal estimates of the number of prey consumed and the size of prey consumed because CFs are not applied to correct for the erosion and partial digestion of otoliths.

Clupeid otoliths are small and fragile and may have been digested and therefore not present in the excretory matter. There was no evidence of salmonids in the diet of seals or cormorants in the Tees Estuary, although individual seals have been observed feeding on salmonids at Tees Barrage were they accumulate until conditions are right to go through the fish pass. These seals may not haul out at Greatham Creek or they may void their faeces before they reach Greatham Creek. Harbour seals have been observed hauling out on the river banks close to the Tees Barrage (Garside, J., Tees Valley Wildlife Trust, pers comm.).

Cormorants roosting at Phillips Jetty have regurgitated otoliths of freshwater fish species so if they were consuming salmonids it would be expected they would regurgitate these remains. Salmonid otoliths and bones are very fragile and it is possible seals would not eat the heads of large prey but if salmonids were a component of seal or cormorant diet it would be expected that there would be some evidence, such as vertebrae.

Some researchers have regarded the presence of invertebrate remains and small fish in seal faecal samples (McConnell *et al*, 1984; Prime and Hammond, 1987) and cormorant pellets (Blackwell and Sinclair, 1995) to be a result of secondary consumption. Secondary digestion of small fish and invertebrates can inflate the actual numbers of prey consumed and over-estimate the proportions of species contributing to the diet. Prime and Hammond (1987) suggested that when large numbers of lesser sandeels are consumed then this represents direct digestion but when small numbers are consumed with larger prey, particularly gadids then they are likely to be secondary. It is possible in this study that the remains of small fish are due to secondary ingestion but they did not consistently occur in the same excretory samples as large prey. The high proportion of 0-1 year old pleuronectids would inflate numbers of prey consumed if they were the result of secondary digestion but have a lesser impact on biomass. Lesser sandeels are only a minor prey item in the harbour seal diet, as are invertebrates other than crustaceans.

This study suggests that Crustacea contribute to the diet of seals and cormorants rather than being secondary consumption, whereas the remains of molluscs and polychaetes in faeces and pellets were assumed to be due to secondary consumption as these invertebrates were consistently present with larger prey species. The main Crustacea remains present in seal faeces were crabs, whereas common shrimp were the main crustacean remains present in cormorant pellets.

2.7. DISCUSSION OF FISH SIZE PREDICTION

Otolith size can be used to predict the length and mass of fish consumed. There are a number of published regression equations for determining fish size from otolith size (Härkönen, 1986; Tollit, 1996; Brown and Pierce, 1998; Leopold *et al*, 2001). There may be regional variation in the relationship between fish size and otolith size, and so determining equations for fish size from fish collected from the region will generate more accurate estimates. Fish body size and otolith size were measured for 10 fish species collected from the intake water of Hartlepool Power Station but the range of otolith sizes obtained were narrower than within the size range of otoliths collected from seal faeces and cormorant pellets for all species in the Tees Estuary, except the sprat. Extrapolation can lead to considerable errors so only sprat size could be predicted using regression equations calculated from known size sprat from the Tees Estuary. In addition, regression equations from published literature used to predict sprat length and mass for known size fish considerably exceeded the actual values whereas they predicted relatively accurate values for fish size of other species. There were 19 fish species identified in seal or cormorant diet that were not present in the Hartlepool Power Station intake water and so published regression equations were used to calculate the fish size for these species.

Otolith length was a stronger predictor of fish length than otolith width for all species. Linear regression accurately predicted fish length using otolith length, whereas the power function was the most accurate method for estimating fish body mass from otolith length. The power equation reflects an angle in the regression line indicating a slight change in the relative growth rate of fish. Increased variation in fish mass may be due to increased differences in relative mass of male and female fish with age, stronger variation in the condition of adult fish compared to juveniles, the difference in mass of adult fish compared to juveniles and the difference in mass of adult before and after spawning (Leopold *et al*, 2001). Tollit (1996) found a two-stage regression equation using predicted fish length to calculate fish mass to be more accurate than a one-stage regression equation predicting fish mass from otolith length. No significant difference in accuracy was found between the two

methods when calculating fish mass from known size fish so the one-stage regression equation was adopted as conducting less calculations reduces error.

Fish size may be under-estimated when predicted from the size of otoliths recovered in the faecal samples due to species-specific erosion and digestion. Species-specific CFs have been calculated from otolith digestion in captive seals to try avoid these under-estimates (Da Silva and Neilson, 1985; Prime and Hammond, 1985; Tollit, 1996; Bowen, 2000), but the use of CFs to determine fish length is unreliable (Bowen, D., Bedford Institute of Oceanography, Dartmouth, pers. comm.). It was observed by Thompson *et al* (1991) that clupeid body size calculated from faeces and actual body sizes of clupeids collected during fish trawls in the Moray Firth were very similar. The seals either selected larger clupeids than captured in trawls and the otoliths were digested to a smaller size or, more realistically, digestion rates reported previously from captive seals were artificially high. CFs for fish size were not applied in this study due to their unreliability and because CFs have only been calculated for otoliths of a few fish species consumed by harbour seals and no otoliths consumed by cormorants. It must be emphasized that there may be species-specific erosion of otoliths and therefore the potential for under-estimates of fish length and mass. The collection of otoliths from known size fish were used to ensure that estimated fish sizes were comparable.

Studies of otoliths recovered from faecal samples indicated that harbour seals consume fish with a range of lengths. Harbour seals off the southeast Shetland coastline consumed fish ranging from 30 to 990 mm (Brown and Pierce, 1998). The majority of fish consumed by harbour seals in the Moray Firth were 100-160 mm in length and although larger cod and herring were consumed, few fish consumed exceeded 300 mm (Tollit, 1996). Harbour seals in the Tees Estuary consumed fish ranging from 12 to 443 mm in length but these seals also preferred smaller fish. The mean mass of whiting consumed was 8.7 to 11.9 g in the Moray Firth (Tollit and Thompson, 1996), whilst the mean mass of whiting consumed on the southeast coastline of Shetland was much greater at 245 g (Brown and Pierce, 1998). The median mass of whiting consumed in the Tees Estuary was 30.3 g. Cod and herring were

the largest species consumed in the Moray Firth, whereas in this study, cod, saithe, poor cod, whiting and herring were the largest fish species consumed. The length of the largest fish from the Tees Estuary was probably under-estimated due to the erosion of the otoliths. The Moray Firth is a nursery area for herring, sprat, *Sprattus sprattus* and small gadids and harbour seals probably exploit this abundance of small prey, explaining the small size of fish consumed. The Tees Estuary is a nursery area but also receives larger fish although not as large as those fish found on the more open coastline areas. Hall *et al* (1998) studied seals in Donna Nook and found that harbour seals did tend to forage on small prey. They concluded that small size of fish taken may simply be a reflection of coastal foraging but it is also consistent with a maximum limit on the preferred size of fish taken by harbour seals. Small prey can be swallowed whole requiring the minimum handling.

Cormorants select prey species and size (Nehls and Gienapp, 1997). The size of fish is influenced by hunger status according to Hustler (1995), with hungry cormorants selecting larger fish. In contrast, Strod (2000) suggested that small fish are caught by hungry cormorants because they are rapidly digested and the slower swimmers in a shoal so more easily caught. Non-starving cormorants will take fish of a range of sizes. Cormorants do consume a range of sizes of fish indicating that food supply is not limited within the Tees Estuary. Seals and cormorants are both opportunists so they would be expected to consume similar sizes of fish when feeding in the same locality. The size of fish consumed by seals and cormorants from the Tees Estuary is relatively comparable despite otoliths being expected to digest less when regurgitated in cormorant pellets than when passed through the digestive system in seal faeces.

Wild cormorants consume about 340 to 520 g of fish per day, constituting 17 to 26 % of the birds' weight (Kirby *et al*, 1996). The typical length of fish caught is 150 to 200 mm in length. Strod (2000) studied prey detection and size preference in captive great cormorants, *Phalacrocorax carbo sinesis*. Foraging underwater by pursuit diving, the great cormorant visually detected prey at a distance of at least 3.1 m in clear water. They tended to take large fish during the first dive and medium size fish on consecutive dives. The cormorants also

chose dead fish significantly more often than live fish. Hustler (1995) conducted prey preference experiments to determine whether two cormorant species, the reed cormorant, *P. africanus* and the whitebreasted cormorant, *P. carbo lucidus*, given a choice, would choose the most profitable size fish. The choice of prey differed significantly from random showing that birds were choosing fish, but not necessarily the size range predicted as most profitable. The hunger status of the cormorants probably influenced the size of fish taken. Smaller fish are more rapidly digested than larger ones so a hungry bird may satisfy its energy demands more quickly by consuming smaller fish. Large fish of the same species swim faster than smaller individuals, as the swimming speed of a fish is an allometric function of its body size and so, in a shoal smaller fish will lag behind and be easier prey targets (Peters, 1983). Non-starving cormorants ate a variety of sizes suggesting they swim fast enough to catch a range of fish. Cormorants roosting in the Tees Estuary consumed a range of fish sizes from 21 to 443 mm in length, although smaller fish were most frequently predated on. This suggests that the cormorants are predating on the prey that are easiest to catch but they consume a range of fish sizes suggesting that sufficient prey is available in the estuary for their energetic demands. Profitability is also affected by factors, such as age and experience of the bird and water depth. Hustler (1995) concluded that whilst cormorants were capable of choosing size of prey their choice was dependent on the feeding conditions.

There was overlap between the length distributions of cod, whiting, saithe, poor cod, pleuronectids and dragonets consumed by both predators, whereas seals consume a greater range of herring body lengths than cormorants. The length frequency distributions for herring and pleuronectids indicated that both predators tended to prefer small individuals of herring and pleuronectids and cormorants preferred small individuals of cod and whiting whereas seals preferred medium size cod and whiting. Seals and cormorants both appear to prefer medium sized saithe, poor cod and dragonet. The foraging behaviour of seals and cormorants was examined in Chapter 3. The intake of metals by seals and cormorants from their diet can be calculated from the biomass of prey consumed multiplied by the metal concentrations in each prey species. The metal concentrations in the main prey species of

seals and cormorants are given in Chapter 4. The biomass of prey consumed may vary seasonally and affect the metal intake by predators. The species and biomass of prey consumed seasonally by these predators was determined in Chapter 3.

Alternative methods of determining prey consumption by pinnipeds include stable isotope and fatty acid signature analyses (Deagle *et al*, 2005). These methods provide less specific, long term data that are useful in many situations but they require the capture of the animals and do not provide detailed information of the taxa and the quantity of prey consumed. Deagle *et al* (2005) used DNA to determine diet consumed by two captive sea lions, *Zalophus californianus*. The proportions of fish DNA present in eight faeces samples were roughly proportional to the mass of the prey items consumed and the authors concluded that this was an accurate method to identify prey species. Parsons *et al* (2005) used this method to identify salmonids prey species in seal faeces. Hard parts remains of salmonids in faeces are easily digested and so the presence of the genera in the diet is often underestimated. Parsons *et al* (2005) concluded that this technique did provide a promising new method for examining prey composition in faeces when implemented alongside conventional prey remains analysis. DNA analysis of faeces has also been used in combination with identification of prey remains in faeces to determine the species and sex of the defecator (Deagle *et al*, 2005).

DNA analysis was a new methodology at the start of this study and its reliability using captive seals had not been fully explored. It is an expensive technique and only a few faecal samples could have been analysed. Hard part analysis is considered sufficiently accurate to estimate the pollutant intake by seals and cormorants in this study, although there may have been an under-estimation of the quantity of clupeids in the diet and the lack of salmonids in the diet may be inaccurate.

CHAPTER 3. FORAGING BEHAVIOUR OF HARBOUR SEALS AND CORMORANTS FROM THE TEES ESTUARY

3.1 INTRODUCTION

The body burdens of metals in prey are expected to differ between species and body size. The species and body sizes consumed seasonally by harbour seals, *Phoca vitulina* and cormorants, *Phalacrocorax carbo* in the Tees Estuary are therefore essential information for determining the intake of metal concentrations by these predators.

3.1.1. Seal foraging behaviour

Harbour seals are principally piscivores and generalist feeders, consuming a wide variety of prey types (Bowen *et al,* 2002). They tend to be opportunistic foragers with a diet that reflects geographical and seasonal availability of prey (Thompson *et al*, 1991). Although pinnipeds may consume a wide variety of available prey, only relatively few species (usually less than five and often only two or three) account for most of the energy ingested in any one season or geographical location (Bowen *et al,* 2002). The mean energetic demand for steady state basal metabolic rate of an adult harbour seals was 130 W (Boyd, 2002). Additionally, energetic demands of a seal are dependent on the distance traveled to feed, which is related to the abundance and location of the prey species. A 70 kg adult harbour seal will expend approximately 39 calories per metre travelled (Härkönen and Heide-Jørgenson, 1991).

Seal foraging behaviour is only observed infrequently. Most prey is consumed underwater, unless it is large and difficult to manipulate. In addition, prey may be caught a long distance from the shore or observation points out to sea. Foraging by the seals on the Seal Sands mudflats in the Tees Estuary was not observed over the 15 year monitoring period (Turner, 2003). Some seal foraging was observed at the Tees Barrage during a summer period of observations but it was infrequent and only for large fish such as salmon, *Salmo salar,* sea trout, *Salmo trutta*, mature flounder, *Platichthys flesus* and eels, *Anguilla anguilla* (Turner, 2003). Foraging observations would not be an adequate method of determining total number and diversity of species consumed and they would be biased towards large fish.

The extent of foraging areas for seals which frequent the Tees is currently unknown. Radio-tracking and satellite telemetry has been used to determine foraging range on the Scottish coast (Thompson *et al*, 1996; 1998). Foraging range can be compared with studies of prey distribution to assess potential prey species. Grey seals, *Halichoerus grypus* have a larger foraging range than harbour seals and tend to consume more offshore prey species, whereas harbour seals tend to consume mainly coastal and estuarine species (Prime and Hammond, 1990). There is overlap in diet preference between the two seal species however, as they are both opportunists and grey seals consume coastal and estuarine species, in addition to offshore species (Thompson *et al*, 1996) Grey seals in the Moray Firth, North-east Scotland foraged up to 145km from their haul-out sites (Thompson *et al*, 1996). The foraging range of harbour seals in the Moray Firth has been reported as being inshore within 30 km of their haul-out site (Tollit, 1996; Thompson *et al*, 1998) and within 60 km of their haul-out sites (Thompson *et al*, 1996). Harbour seals studied by Thompson *et al* (1998) in Scotland had summer foraging ranges of 4 to 55 km. The duration and range of foraging were significantly shorter for females. Tollit (1996) recorded that most harbour seal dives were benthic, to depths of 10-50 m, and foraging was mainly amongst sandy-seabed sediments. Occasional pelagic dives were made. Radio-tracking was not seen as appropriate for this study as it is expensive, time-consuming and limited to a few individuals that may not be representative of the population. As long-lived predators, most species of marine mammals exhibit individual foraging specializations (Bowen *et al*, 2002), although these specializations are not presently understood for harbour seals. It would therefore be necessary to gather data from a large number of animals to be accurate. In addition, the seals would need to be captured to fit the equipment and it was desirable in this study to avoid disturbance and information on the species and size of prey eaten could not be determined.

A number of studies have used faecal analysis to study the diet of harbour seals. A number of studies of harbour seal faecal samples in the Moray Firth reported the pre-dominance of lesser sandeels, *Ammodytes tobianus,* gadids, clupeids, pleuronectids and salmonids in the

diet (Pierce *et al*, 1991; Thompson *et al*, 1991; Tollit, 1996; Tollit and Thompson, 1996; Tollit *et al*, 1997a; Brown and Pierce, 1997; 1998). Forty harbour seal faecal samples collected in the Moray Firth contained at least 491 individual fish and seventeen fish species (Thompson *et al*, 1991). Clupeid otoliths and bones were found in 75% and 90% of samples, respectively. The other species occurring most frequently were cod, *Gadus morhua* and flounder, other gadid species, ammodytids and gobids. This diet reflected trawl data in the area. Brown and Pierce (1997; 1998) studied the diet of harbour seals foraging in the Moray Firth and along the southeast coastline of the Shetlands. Sandeel otoliths were most numerous, followed by Gadidae. By biomass, Gadidae, particularly whiting, *Merlangius merlangus*, accounted for an estimated 53.4% of the diet, lesser sandeels 28.5% and pelagic fishes 13.8%.

The only study of seal diet conducted on the Tees Estuary was undertaken in September-October, 1989 on a predominately grey seal haul-out on Seal Sands (Wilson, 1994). The twenty-two faecal samples mainly contained small cod, whiting, haddock, *Melanogrammus aeglefinus,* flounder, dab, *Limanda limanda* and lesser sandeel. Five faecal samples only contained Crustacea remains.

Comparison of harbour seal diet from different areas of the North coast of Britain indicate that seals consume a similar range of species. There is variation however, in the proportions of each species within the seal diet and the seasonal consumption of species. Pierce *et al* (1990) found regional differences in the diets of both common and grey seals between Orkney, Isle of May and the Moray Firth. Tollit (1996) observed variations in harbour seal diet in the Moray Firth that appeared to relate to local differences in foraging habitat preferences. Tollit and Thompson (1996) found that harbour seal diet in the Moray Firth varied in relation to local changes in food availability, especially over-wintering clupeids. Tollit (1996) stated that seals adjust their foraging patterns to take advantage of local and seasonally abundant prey. They will consume dermersal, pelagic, schooling and solitary fish, cephalopods and Crustacea. Tollit *et al* (1997a) found that when shoaling pelagic fish were abundant they were the dominant prey species for harbour seals foraging in the Moray

Firth but when the abundance of these prey species was low, diet preference switched to benthic species. A number of studies indicate seal preference for small, abundant, aggregated prey species (Boulva and McClaren, 1979; Pitcher, 1980; Bowen and Harrison, 1996, Tollit, 1996). The potential benefits of feeding on this prey type include high encounter and capture rates. Small fish can be swallowed whole and require the minimum of handling. Small, schooling fish, such as herring, *Clupea harengus* and lesser sandeels, tend to have relatively high energy densities (Murray and Burt, 1977; Hislop *et al*, 1991).

Seals are opportunistic foragers and therefore their diet tends to reflect seasonal availability of prey (Thompson *et al*, 1991). Prey species provide different calorific values and hence, seasonal and regional variations in seal foraging behaviour will affect energy requirements and total food consumption (Stephens and Krebs, 1986). Dietary changes in relation to prey abundance and net energetic benefits of feeding on different prey need to be understood (Pyke, 1984). In addition, different prey species may accumulate different metal concentrations and seasonal variation in diet in the Tees Estuary will influence metal uptake by seals. A number of studies have assessed seasonal diet variations (Tollit and Thompson, 1996; Hall *et al*, 1998). There were strong seasonal patterns to the contribution of lesser sandeels and gadids in harbour seal diet in the Moray Firth (Tollit and Thompson, 1996). Lesser sandeels were the dominant prey in March to June and gadids dominant in the diet for much of the rest of the year. Pelagic species (mainly herring, garfish, ***Belone belone*** and mackerel, *Scomber scombrus*) were an important prey ecotype during the summer, although their importance was possibly under-estimated due to erosion of their fragile otoliths during passage through the gut. There was strong seasonal variation in harbour seal diet in the Wash, on the east coast of England (Hall *et al*, 1998). Whiting, bib, ***Trisopterus luscus*** and bullrout, *Myoxocephalus scorpius* dominated from late autumn through early spring; sand goby, *Pomatoschistus minuta* peaked during winter and early spring; dragonet, *Callionymus lyra*, lesser sandeel and flatfish (except sole, *Solea solea*) dominated from late spring to early autumn and sole peaked in the spring. This strong seasonality in diet appeared to be linked mostly to prey availability, with whiting, dab and plaice, *Pleuronectes platessa* consumption appeared to be related to the availability of other species.

3.1.2 Cormorant foraging behaviour

Cormorants are opportunistic, primarily piscivorous feeders, exploiting a range of fish species according to season and locality, including rivers and lakes as well as estuaries and coastal marine environments (Cramp and Simmons, 1977). Their diet is therefore subject to spatial differences and temporal shifts. Cormorants feed exclusively in daylight hours, diving to or near to the sea bottom and returning to the surface to swallow prey captured. Cormorants rarely dive deeper than 10 m (Nelson, 1980). The average dive depth is 1-3m and the time of the dive varies between 15-60 seconds (Cramp and Simmons, 1977). The male has a greater bill depth than the female, so is able to take larger prey (Koffijberg and Vaneerden, 1995). Males consumed smelt, *Osmerus eperlanus* and eel that were 7% and 21% larger than those consumed by the female, respectively.

Foraging in cormorants is difficult to observe because these foot-propelled pursuit divers often consume fish underwater (Gremillet, 1997). Gremillet *et al* (1998) used radio-tracking to study the flexible foraging techniques in breeding cormorants at the Chausey Islands in France. The cormorants fed exclusively on pelagic fish during social fishing (5% of trips) and executed 11% pelagic, 60% benthic and 29% intermediate dives during solitary trips (95% of trips). The proportions of benthic to pelagic dives varied widely between dive sequences of single birds and between individuals and gender.

Several researchers have used analysis of regurgitated pellets to determine the prey species and size of prey consumed by cormorants (Kirby *et al*, 1996; Russell *et al*, 1996; Gremillet *et al*, 1998; Leopold *et al*, 1998). In coastal areas, diet is primarily benthic fish with invertebrates as a small, but consistent portion of the diet. A combination of six studies around the British and Irish coasts recorded a very wide potential prey spectrum of over 30 marine species of 22 dermersal and two pelagic families and six species from freshwater sites (Russell *et al*, 1996). Some component studies recorded consumption of up to 16 different species. Commercial fish including gadids and flatfish were important prey items. Kirby *et al* (1996) reported that prey caught in coastal waters included flatfish, a variety of

other marine fish, eels and some salmonids. The diet of cormorants in the Dutch Waddensea was estimated from otoliths found in 182 regurgitated pellets collected at the main night roosts and in one colony (Leopold *et al*, 1998). Otoliths of at least 24 different species were found with flatfish representing 73% of fish numbers and 79% of fish biomass. Plaice were most numerous (46%), followed by dab (34%), flounder (19%) and sole (1%). Goutner *et al* (1997) studied the diet and growth of cormorant nestlings in the Mediterranean estuarine environment from regurgitates and also found a dominance of benthic fish. Changes in numbers and wet biomass of prey composition during the study were thought to result from opportunistic foraging behaviour. Gremillet *et al* (1998) analysed 526 pellets containing 13,016 otoliths at the Chausey Islands in France. The cormorants fed on at least 22 different fish species. These fish species were predominately benthic (67%) but 29% were pelagic, confirming that cormorants are flexible foragers. Nehls and Gienapp (1997) used a combination of pellet analysis and direct observation to determine that the diet of cormorants in the Wadden Sea was dominated by young fish of the year, especially flatfish. Prey species of cormorants consisted of 15 fish and three invertebrate species. The fifteen fish species were plaice, dab, flounder, sole, cod, whiting, butterfish, *Pholis gunnellus*, lesser sand-eel, grey gurnard, *Eutrigla gurnardus,* herring, eel, sand goby, common goby, *Pomatoschistus microps,* Nilsson's pipefish, *Syngnathus rostellatus* and bullrout, *Myoxocephalus scorpius*. The three invertebrate species were shore crab, *Carcinus maenas,* common shrimp, *Crangon crangon* and king ragworm, *Neanthes virens*.

Richner (1995) assessed the affects of seasonal, diurnal and tidal variables on the wintering foraging of cormorants in the Ythan Estuary, Scotland. One to two year old flounder (10 to 20 cm long) constitute 85% of the cormorant diet in this region. The highest numbers of cormorants in the Ythan Estuary occurred in October and dropped significantly (by over 70%) to a minimum in January and February before increasing again at the beginning of March. This seasonal population change in cormorants correlates with and may be attributable to the seasonal abundance of flounder.

3.2 METHODOLOGY FOR DIET DETERMINATION IN HARBOUR SEALS AND CORMORANTS

3.2.1. Diet indices

There are a number of diet indices that can be used to determine prey consumption but none give a complete or fully realistic picture of dietary composition on their own (Berg *et al*, 2002). Three methods were used to compare the total composition of prey consumed and the seasonal composition of prey consumed by harbour seals and cormorants: relative numerical frequency of prey consumed, the frequency of occurrence of each species and the biomass.

These three methods were used in combination to provide as accurate an account of diet as possible. Counts of numerical frequency show the overall range of species consumed but tend to over-estimate the importance of numerous prey species and under-estimate contribution of larger, less common prey (Hyslop, 1980). One pellet may contain a large number of otoliths from small individuals or only one or two otoliths from large fish but the total biomass of the meal would be comparable. This is particularly problematic if small prey were present due to secondary consumption. Frequency of occurrence of prey is a less biased method of determining the main species consumed. Prey biomass combines the number and size of the fish species consumed. This method is considered the most appropriate to illustrate differences in diet composition (Pierce and Boyle, 1991; Hammond *et al*, 1994, Tollit, 1996) and the intake of metals by these predators from their diet can be calculated from the biomass of prey consumed multiplied by the metal concentrations in each prey species given in Chapter 4. The biomass of prey consumed and metal concentrations may vary seasonally so the biomass of seasonal prey consumed is shown.

Fish biomass can be estimated relatively accurately, although precision may be low. Bias can be reduced by analysing large sample sizes and determining the prey frequency of occurrence in addition to biomass. Biomass determines the total mass of prey consumed and does not indicate of the number of prey items consumed. It therefore can not be used as a measure of occurrence. The effect of secondarily ingested prey when using diet indices

such as frequency of occurrence or biomass are used is negligible. It was not possible to estimate the biomass of crustaceans consumed from the hard parts because they were often fragmented. Crustacea biomass was estimated by multiplying the median biomass of common shrimp and shore crab collected from the Hartlepool Power station by the number of each species that was counted in the excretory matter of seals and cormorants.

The relative frequency of individual prey consumed can be estimated by dividing the total number of otoliths, premaxillae, preoperculum and pharyngeal teeth in each faeces or pellet by two and rounding up to an integer (Pierce *et al*, 1991). This method assumes that where hard parts occur in pairs, both hard parts from each individual fish consumed will be present in each faeces or pellet and will not become separated. This is likely to be the case in cormorants since in captive trials they produced one pellet per day independent of the number of meals or species of fish consumed (Zijlstra and Vaneerden, 1995), but for seals it is an assumption that may lead to an over-estimate. One fish species was counted for between 40 and 60 vertebrae depending on the species. One individual crustacean or mollusc was counted where whole exoskeletons and shells were recovered. Where it was not possible to count the number of individuals represented by exoskeleton and mollusc shell fragments the presence of one individual was noted. The number of individual Crustacea and polychaetes consumed were estimated by dividing the total number of chelae or jaws in each faeces or pellet by two and rounding up to an integer

The frequency of occurrence is the number of samples containing remains of one or more individuals of each prey type, expressed as a percentage of the number of samples. Frequency of occurrence for any given species or group (FO_k) was determined by the equation :

$$FO_k = (NO_k/NS) \times 100$$

Where NO is the number of samples containing species k and NS is the number of samples with otoliths.

FO_k does not provide an indication of the relative amounts of the different prey types or the prey size because percentages are summed across all prey types, so they exceed 100% (Hyslop, 1980). 'Modified frequency of occurrence' (MFO_k) calculates a percentage out of 100%. It was the equation used in this study as it provides a more comparable calculation, determining relative amounts of different prey types or prey size (Bigg and Perez, 1985). MFO_k indicates prey consumption without regard to other prey, and can be used to indicate temporal availability, selectivity or ease of capture of individual prey (Arim and Naya, 2003).

$$MFO_k = (FO_k/100) \times \sum FO_k$$

3.2.2. Statistical analysis

The biomass of prey will be multiplied by metal concentrations in prey (Chapter 4) to calculate the daily metal burden taken in by seals and cormorants from their diet (Chapters 6 and 7). It is therefore important to determine whether the biomass of prey consumed is different between seasons and between predators to identify whether separate calculations are required for seasons and each predator.

Body mass of prey was not significantly different from a normally distribution so parametric statistical tests could be used. Means were compared and the one-way ANOVA was used to test for difference between the seasonal biomass of total prey consumed by each of seals and cormorants. The least significant difference (LSD) *post hoc* tests were used to discern which pairs of bi-monthly periods were significantly different. The independent t-test was conducted to test for difference between biomass of total prey consumed by seals and cormorants. The two-way ANOVA was used to test for difference between the total prey and the main prey groups consumed by seals and cormorants for each bi-monthly period. The least significant difference (LSD) *post hoc* tests were used to discern which pairs of bi-monthly periods were significantly different.

Costello graphics were used to pictorially comparison the dominance of the prey items in seal and cormorant diet (Marshall and Elliott, 1997). The % occurrence is plotted against

the % mass for each of the main prey species (10 or more items) and then interpreted with respect to the position on the graph. Rare prey will be positioned in the bottom left hand corner of the graph and dominant prey will be positioned in the top right hand corner. The feeding stategy of the two predators can also be compared. Prey positioned in the top left hand corner of the graph indicate specialization and prey positioned in the bottom right hand corner indicate a generalist feeder. The importance of the prey in the seal and cormorant diet was ranked using the index of preponderance (I_p) :

$$I_p = V_i O_i / \sum (V_i O_i)$$

Where V_i and O_i are % weight and occurrence respectively (Marshall and Elliott, 1997).

The Shannon-Wiener index, as a summary of the diversity of prey organisms encountered (Marshall and Elliott, 1997), was used to give an indication of niche breadth for seal and cormorant diet for each bi-monthly period. The Shannon-Wiener information statistic (H') and evenness index (J), the standardized value of H' are :

$$H' = \sum P_i \ln P_i$$

$$J = H' / H'_{max}$$

respectively, where P_i = proportion of the observations found in category i and H'_{max} = ln (k), the maximum possible diversity for a set of data consisting of k categories (Marshall and Elliott, 1997).

TWINSPAN was used to describe the similarities in diet between cormorants and seals. This ordination technique orders the samples i.e. the predators according to their food attributes i.e. prey abundance. An ordered two-way array of both samples and attributes is produced (Marshall and Elliott, 1997). The raw data was transformed by the data in the column

$$T = X_{ij} / ((\text{sum}X_i \times K) \times 1/K$$

Where T = transformed data, X = original raw data and K = column total. The cut levels were divided by the frequencies 0.0005, 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1 and 0.2.

The main prey groups consumed were grouped into small, medium and large prey (Table 3.1).

Table 3.1. Grouping of body sizes for the main prey items consumed by seals and cormorants from the Tees Estuary

	Small (g)	Medium (g)	Large (g)
Herring	0 - 50	51 -150	151 +
Cod	0 - 200	201 - 600	601 +
Whiting	0 - 50	51 -150	151 +
Saithe	0 - 100	101 -200	201 +
Poor cod	0 - 50	51 -150	151 +
Pleuronectids	0 - 50	51 -150	151 +

3.3. RESULTS OF FORAGING BEHAVIOUR OF HARBOUR SEALS

Over three-quarters of the total 175 faecal samples collected from Greatham Creek mudflats between June 1999 and June 2003 contained skeletal parts (Appendix F). Visits were made to the haul out site fortnightly to collect the faecal samples. There was considerable variation in the number of faecal samples collected during each season ranging from 16 in September - October to 42 in May - June. The number of harbour seals hauling out on Greatham Creek ranged from none on some cold days in November to February to 55 seals observed on a hot day in August.

The total number of sagittal otoliths in the faecal samples divided by two and rounded to the lowest integer estimates was 880. Ten faecal samples contained other fish bones for 26 individuals but no otoliths. In addition, one faecal sample contained two sprat bones and whiting otoliths and one faecal sample contained four plaice bones but only whiting otoliths. It is possible that the sprat and plaice were consumed by the whiting and the otoliths had digested but this can not be proven so was discounted. These fish bones increased the estimated number of fish consumed to 906. Identification from fish bones other than otoliths therefore only increased the number of fish consumed by a total of 3.0% (Appendix Gi). These included; 21 clupeids, two gadids, two pleuronectids and one dragonet. The number of prey remains found per season varied (Table 3.2).

Table 3.2. Seasonal variation in skeletal remains found in harbour seal faeces collected from Greatham Creek, June 1999- June 2003 (Data from replicate months are combined)

Month	No. of otoliths/2	No. of other fish skeletal remains	Invertebrate parts	Total prey	No. of faeces with prey present	Mean no. of prey per faeces with prey present
Jan-Feb	121	0	21	142	22	6.45
Mar-Apr	137	1	20	158	16	9.88
May-June	80	0	59	139	30	4.63
Jul - Aug	143	5	47	195	32	6.09
Sept- Oct	104	18	74	196	19	10.32
Nov- Dec	295	2	12	309	19	16.26
TOTAL	**880**	**26**	**233**	**1139**	**138**	**8.25**

There is an assumption that all hard parts from a given fish are present in one faeces but they may be excreted in several faeces leading to an over-estimate of fish consumed. The number of prey consumed was estimated in Chapter 2 but due to the potential for otoliths to erode these are minimal estimates, particularly species with small, fragile otoliths, such as the clupeids.

The main Crustacea remains recovered were chelae and exoskeleton remains. Other invertebrate parts identified were mollusc shell and *Nereidae* jaws. Two hundred and thirty-three invertebrate hard parts were identified in 46 seal faecal samples. These invertebrate remains comprised 12 common shrimp, *Crangon crangon*, 41 crab, 13 ragworm, 121 gastropods and 44 bivalves. The seven faecal samples containing invertebrate remains and no fish remains, were regarded as evidence of independent intake of invertebrates. They comprised of two faecal samples containing one common shrimp each, three faecal samples containing the remains of three crabs and one faecal sample containing the remains of two crabs and a scrobicular shell. All other invertebrate remains were present with the skeletal remains of either gadids or pleuronectids, with the exception of one *Littorina* shell found with clupeid remains and one scrobicular shell found with crab remains. It was assumed that the seals had directly consumed the common shrimp and crabs, whilst the presence of other invertebrate remains were a result of either secondary consumption or accidental ingestion whilst foraging on the benthos. Common shrimp and crab remains accounted for 5.15% and 17.60% of invertebrate remains, respectively. The greatest number of invertebrates consumed was in September to October (Figure 3.1). This was also the period of the highest count of seven common shrimp (58% of total common shrimp consumed). The highest count of 22 crabs was consumed in May to June (54% of crabs consumed).

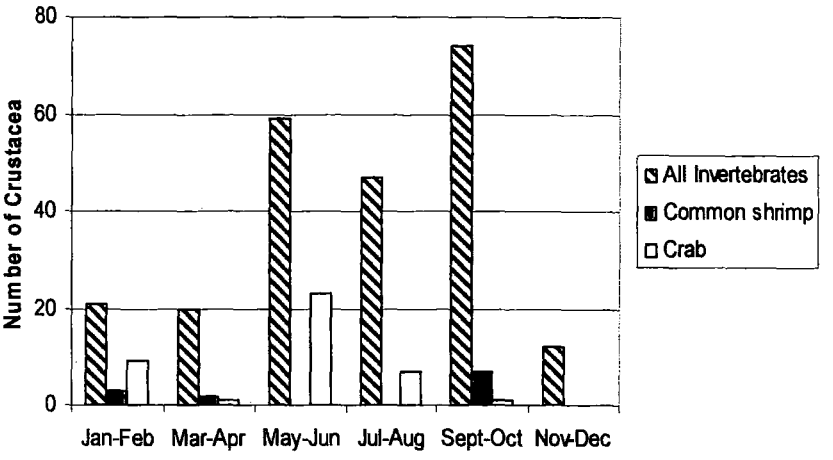


Figure 3.1. Seasonal numbers of Crustacea and other invertebrates present in harbour seal faecal samples, June 1999 - June 2003

The number of faeces containing remains of each prey and the relative numerical frequency, the modified frequency of occurrence and the biomass of the harbour seal diet were compared (Table 3.3).

Table 3.3. Number, occurrence and biomass of prey species in harbour seal faecal samples from the Tees Estuary, June 1999 - June 2003 (N.C. = not calculated)

Species	No. of faeces	Relative numerical frequency	MFOk	Total biomass (g)
Clupeiformes	29	87	21.0	848.6
Herring	13	21	9.4	600.5
Sprat	21	66	15.2	248.1
Gadiformes	77	556	56.6	44727.5
5 bearded rockling	3	6	7.3	72.4
Cod	39	101	28.8	13627.3
Haddock	1	1	0.7	4.2
Whiting	45	317	33.6	6677.2
Saithe	10	15	2.2	561.4
Poor cod	24	116	28.8	23785.1
Perciformes				
Lesser weever	2	3	1.5	13.4
Eelpout	3	4	2.2	N.C.
Lesser sandeel	2	12	1.5	150.8
Dragonet	5	21	3.6	277.9
Pleuronectiformes	54	1084	38.4	4660.2
Crustacea				
Common shrimp	8	12	5.6	27.6
Shore crab	17	41	11.8	930.7

Pleuronectids were numerically the most important prey, whereas Gadids occurred most frequently in faecal samples and were the most important prey in terms of biomass. The most frequently consumed gadids were whiting, cod then poor cod, *Trisopterus minutus,* whereas cod, whiting then poor cod constituted the highest biomass of gadid species. Sprat were the most frequently consumed clupeid species and the fifth most frequently consumed species but in terms of biomass clupeids composed only a small part of the diet.

3.4 RESULTS OF FORAGING BEHAVIOUR OF CORMORANTS

A total of 360 cormorant pellets were collected between January 2000 and December 2002 from Phillips Jetty, Seal Sands and 333 (92.5%) contained otoliths, 35 contained other fish skeletal remains and no otoliths and eight pellets contained only invertebrate remains with no fish. Prey remains, either fish or invertebrate, were therefore present in 356 pellets.

There was seasonal variation in the number of prey present in the pellets (Table 3.4). The total number of sagittal otoliths in the pellets divided by two and rounded to the lowest integer estimates was 3190. These fish were identified at least to order and mostly to species. Some pellets did not contain otoliths but contained other fish bones. There were 35 fish identified from other bones when otoliths were absent, increasing the total consumption to 3225 fish. Where otoliths and fish bones from the same species were present in a faecal sample this was assumed to be the same individual. The use of skeletal remains other than otoliths increased the number of fish consumed by cormorants by only 0.93% overall but they were important in the detection of 6 clupeids, 2 eelpout, 1 bullrout, 9 dragonet, 1 butterfish, 14 cyprinids, 1 lesser weever and 1 lesser sandeel (Appendix Gii). Related species are more difficult to distinguish than broader taxonomic groups and using both otoliths and other skeletal parts in combination reduces the likelihood of misidentification at species level.

Table 3.4. Seasonal variation in skeletal remains found in cormorant pellets collected from Seal Sands, January 2000-December 2002 (Data from replicate months are combined)

Month	No. of otoliths/2	No. of other fish skeletal remains	Invertebrate parts	Total prey	No. of pellets with prey present	Mean no. of prey per pellet with prey present
January	369	8	42	419	58	7.22
March	544	2	67	613	59	10.39
May	753	7	124	884	60	14.73
July	520	7	89	616	59	10.44
September	590	6	174	770	60	12.83
November	414	5	34	453	60	7.55
TOTAL	**3190**	**35**	**530**	**3755**	**356**	**10.55**

There is an assumption that all hard parts from a given fish are present in one faeces but they may be excreted in several faeces leading to an over-estimate of fish consumed. The number of prey consumed was estimated in Chapter 2 but due to the potential for otoliths to erode these are minimal estimates, particularly species with small, fragile otoliths, such as the clupeids.

The number of invertebrates present is an estimate based on the identification methods given in the methods of Chapter 2. Eight pellets contained only invertebrate remains and no fish remains. Common shrimp remains were present in each of these eight pellets. Remains of two molluscs and one ragworm, *Nereis diversicolor* were present in one of these pellets with common shrimp remains but no fish remains. Cormorants in the Tees Estuary therefore appear to directly consume common shrimp. Fragments of crab were found in cormorant pellets without fish remains in 22 pellets and just three pellets contained crab and fish remains therefore it was assumed that crab remains were also the result of direct consumption. The size of crab consumed is expected to be limited because cormorants swallow their prey whole. Common shrimp and crab remains accounted for 27% and 6% of total invertebrates consumed, respectively. The remaining 67% of invertebrates comprised molluscs and ragworm remains were assumed to be the result of secondary or accidental ingestion.

There was seasonal variation in the number of invertebrates consumed (Table 3.5). The greatest number of invertebrates consumed was in September to October. This was also the period of the highest count of 40 common shrimp (28% of total common shrimp consumed). The highest counts of 10 (54%) and 9 (54%) crabs was consumed in January-February and May to June, respectively.

Table 3.5. Seasonal numbers of invertebrates present in cormorant pellets collected from Seal Sands, January 2000-December 2002

Species	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sept-Oct	Nov-Dec	Species totals	% of total
No. of invertebrates	42	67	124	89	174	34	530	
No. of C. shrimp	14	35	15	23	40	16	143	26.98
No. of S. crab	10	3	9	3	3	4	32	6.04

The number of pellets containing remains of each prey and the relative numerical frequency, the modified frequency of occurrence and the biomass of the total cormorant diet were compared (Table 3.6). Pleuronectids were numerically the most important prey, followed by gadids. In contrast, the most frequently consumed prey and the dominant prey groups by percentage biomass were gadids, followed by pleuronectids although pleuronectids occurred most frequently and constituted the greater biomass when compared to individual gadid species. The most frequently consumed gadid species and the main fish species consumed by biomass were whiting, cod and saithe, *Pollachius virens*. The most frequently consumed clupeid species were herring.

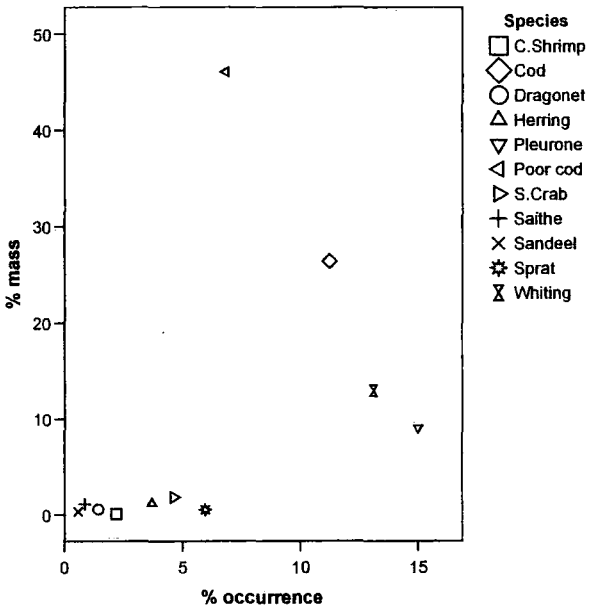
Table 3.6. Number, occurrence and biomass of prey species in cormorant pellets collected from Seal Sands, January 2000-December 2002 (N.C. = not calculated)

Species	No. of pellets	Relative numerical frequency	MFOk	Total Biomass (g)
Clupeiformes	19	35	7.5	2191.2
Herring	17	33	6.1	2169.5
Sprat	2	2	1.5	21.8
Gadiformes	231	1702	66.8	65081.3
5 Bearded rockling	11	26	3.2	615.7
Cod	110	436	31.8	18598.1
Haddock	18	72	5.2	3414.3
Whiting	139	887	40.2	28654.9
Saithe	67	204	19.4	5842.3
Poor cod	17	77	4.9	7956.0
Scorpaeniformes				
Bullrout	18	40	5.8	3647.5
Grey gurnard	2	4	0.6	132.8
Perciformes				
Scad	13	35	3.8	3982.8
Wrasse	2	9	0.6	274.5
Lesser weever	90	1408	26.3	7912.6
Eelpout	3	9	1.5	63.3
Butterfish	1	2	0.6	41.6
Lesser sandeel	33	388	9.8	3822.8
Dragonet	48	173	16.2	3557.9
Pleuronectiformes	206	2210	59.5	40584.7
Freshwater				
Perch(Perciformes)	17	115	4.9	5366.0
Roach (Cypriniformes)	21	247	10.1	9799.1
Crustacea				
Common shrimp	77	144	17.1	331.2
Shore crab	25	32	7.2	726.4

3.5. COMPARISON BETWEEN HARBOUR SEAL AND CORMORANT FORAGING BEHAVIOUR IN THE TEES ESTUARY

The dominance of prey items and the feeding strategy of harbour seals and cormorants were compared using Costello graphics (Figure 3.2).

a)



b)

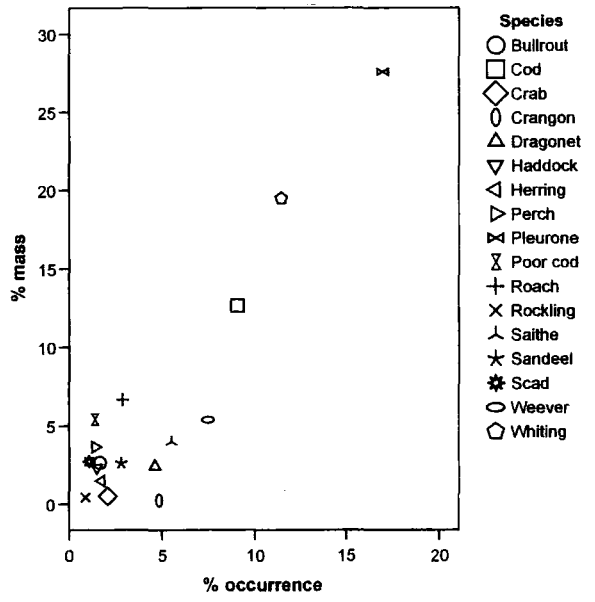


Figure 3.2. The importance of prey items in the diet of a) harbour seal and b) cormorant, and feeding behaviour of each species, as determined by the Costello analysis

The dominant prey species consumed by harbour seals were poor cod and cod by weight and pleuronectids, whiting and cod by occurrence. The dominant prey species consumed by cormorants by weight and occurrence were pleuronectids, then whiting and cod. The postioning of the points indicate that a few dominant species are common in the diet of both predators but each predator also takes other prey species on occasions and both predators have a feeding strategy that is intermediate between a specialized and a generalist diet.

The index of preponderance produces a ranking of the main prey items in the diet of harbour seals and cormorants from the Tees Estuary (Table 3.7).

Table 3.7. Ranking and values of the main prey items in the diet of harbour seals and cormorants from the Tees Estuary, as determined by the index of preponderance

Harbour seal		Cormorant	
Prey	I_p	**Prey**	I_p
Poor cod	0.3363	Pleuronectids	0.5004
Cod	0.3194	Whiting	0.2383
Whiting	0.1825	Cod	0.1224
Pleuronectids	0.1455	Weever	0.0431
Shore crab	0.0089	Saithe	0.0234
Herring	0.0046	Roach	0.0205
Sprat	0.0031	Dragonet	0.0119
Saithe	0.0010	Poor cod	0.0081
Dragonet	0.0008	Sandeel	0.0078
Rockling	0.0004	Perch	0.0055
Sandeel	0.0002	Bullrout	0.0046
Common shrimp	0.0001	Haddock	0.0037
		Scad	0.0031
		Herring	0.0027
		Common shrimp	0.0012
		Shore crab	0.0011
		Rockling	0.0004

Cormorants exhibited a greater niche breadth of diet than seals. Cormorants consumed 17 main prey species, whereas seals consumed 12 main prey species and cormorants consumed a greater range of less frequently consumed prey. Gadids were the dominant prey items in the diet of seals with poor cod being most dominant, followed by cod and whiting, whereas pleuronectids were the most dominant prey items in the cormorant diet followed by whiting, then cod. Poor cod were the most dominant prey item by weight in the seal diet but

they were not a dominant prey item in cormorant diet. Shore crab and clupeids were more prevalent in seal diet than in cormorant diet, where they were rare prey items. Weever was the fourth most dominant prey item in the cormorant diet but was not included in the main prey items consumed by seals. Dragonets were more prevalent in cormorant than seal diet.

A comparison of niche breadth was achieved through the use of the Shannon-Wiener index and associated evenness index (Table 3.8). Harbour seals had a more specialized diet with a smaller number of species consumed than cormorants for all bi-monthly periods, except May to June, and over all months. Harbour seal diet showed the most diversity in May to June whereas cormorant diet, in contrast, was most specialized in May to June. Cormorant diet showed the most diversity in terms of the number of species taken in the winter months.

Table 3.8. Shannon-Wiener H' and evenness, J, indices indicating the niche breadth of seal and cormorant diet, seasonally

		Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sept-Oct	Nov-Dec	Overall
Seal	**H'**	1.39	0.72	1.90	0.88	1.26	0.72	1.40
	J	0.49	0.25	0.67	0.31	0.45	0.25	0.49
Cormorant	**H'**	2.12	1.87	1.36	1.77	1.82	2.01	2.04
	J	0.65	0.57	0.42	0.54	0.56	0.62	0.70

The similarities between the diet of harbour seals and cormorants are shown using TWINSPAN analysis to produce a dendrogram (Figure 3.3). The species at the top of the dendrogram are those that were most commonly consumed by seals and those at the bottom were species most commonly consumed by cormorants. Large herring were only consumed by seals and roach and perch were only consumed by cormorants. Sprat were mainly consumed by seals and weever were mainly consumed by cormorants. The prey species in blue text in middle of the dendrogram were consumed in comparable quantities by both predators.

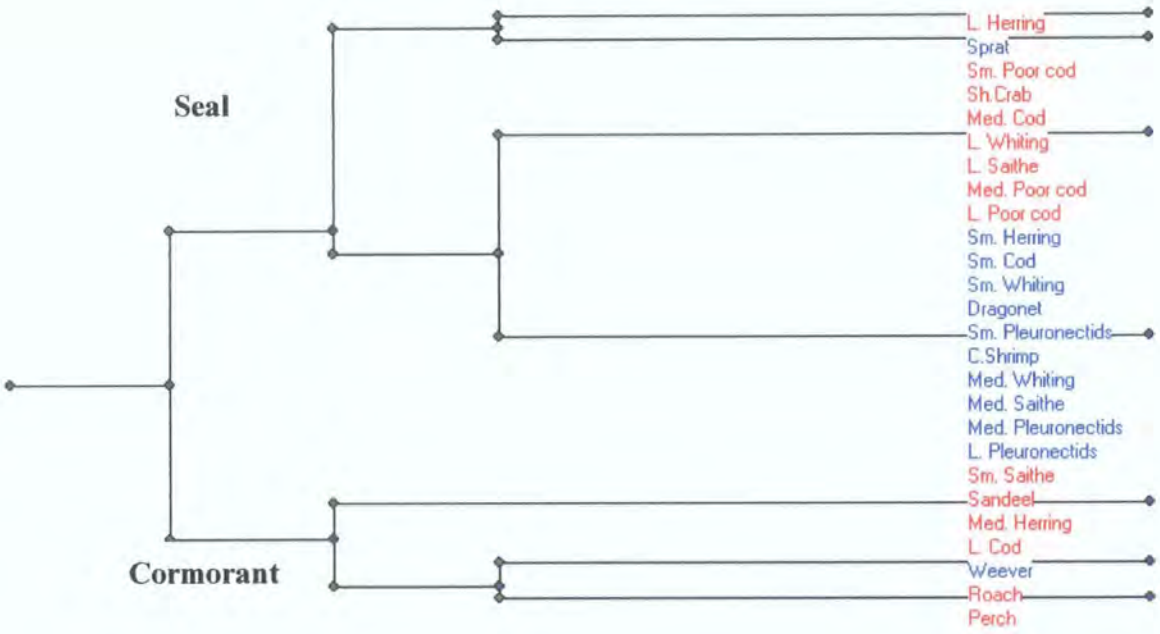
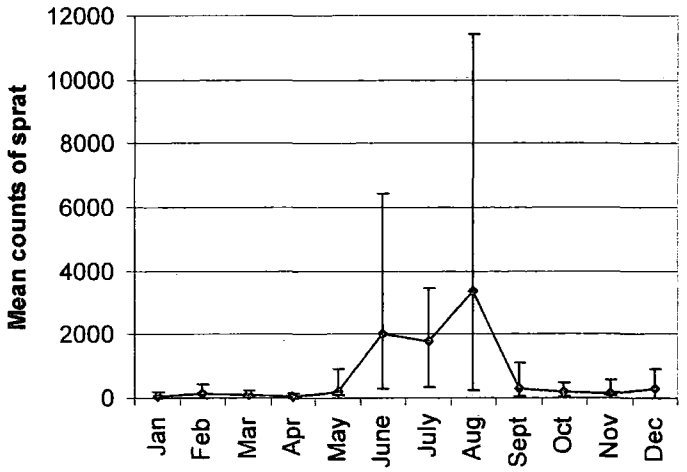


Figure 3.3. A dendrogram showing the dietary comparisons of seal and cormorant diet using the TWINSPAN analysis.

To determine if the seals and cormorants consume seasonally available prey or select prey by preference the seasonal main prey species counted in the Hartlepool Power Station intake water by the Environment Agency (EA) (Chapter 1) were compared with the seasonal numbers of prey consumed by seals and cormorants (Figures 3.4 – 3.7). Sprat and herring numbers peaked in the counts from the Hartlepool Power Station in June to August, whereas herring were most frequently consumed by seals in July to August and sprat were most frequently consumed by seals in July to October. Sprat and herring were rarely consumed by cormorants so the seasonal consumption was not shown. Whiting numbers peaked in the counts from the Hartlepool Power Station and the seasonal consumption by cormorants during the winter months but they were most frequently consumed by seals in March to April. The number of flounder in the counts from the Hartlepool Power Station peaked twice in June to July and September to October, whereas they were most frequently consumed by cormorants in July to October and most frequently consumed by seals in July to February, peaking in November to December.

a)



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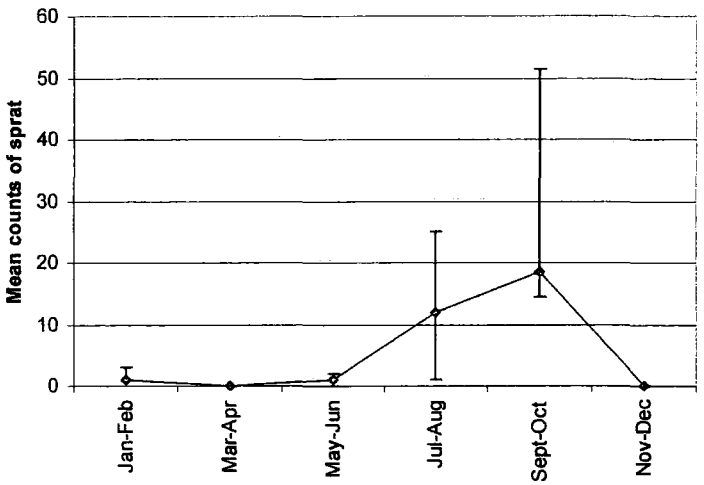
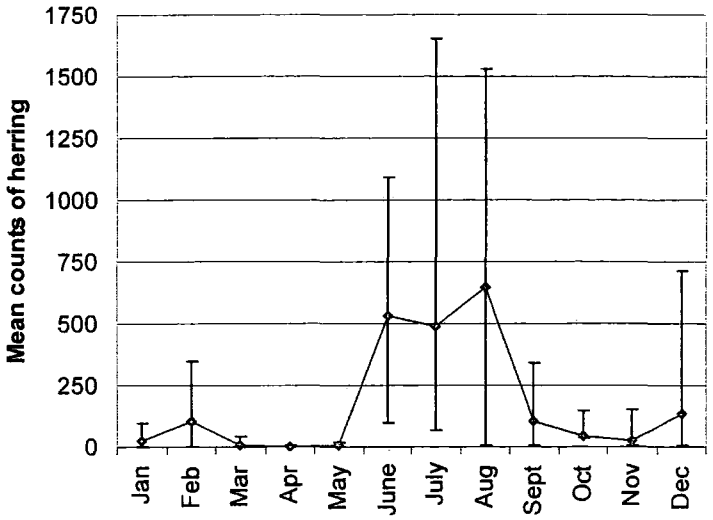


Figure 3.4. Comparison of seasonal changes in abundance (mean and range) of sprat counted in a) intake water screens of Hartlepool Power Station, Tees Estuary, 1992-2002 (Calculated from raw data provided by D. Bastreri, Environment Agency, 2002) with b) the seasonal number of sprat consumed by harbour seals from the Tees Estuary

a)



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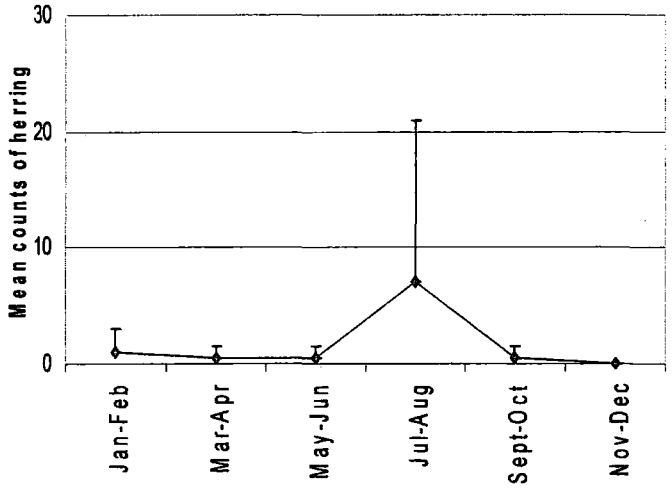
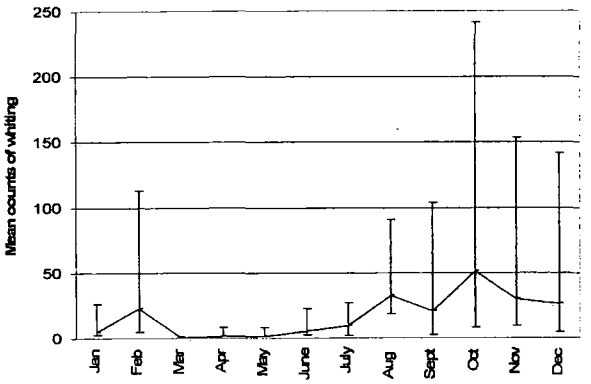


Figure 3.5. Comparison of seasonal changes in abundance (mean and range) of herring counted in a) intake water screens of Hartlepool Power Station, Tees Estuary, 1992-2002 (Calculated from raw data provided by D. Bastreri, Environment Agency, 2002) with b) the seasonal number of herring consumed by harbour seals from the Tees Estuary

a)



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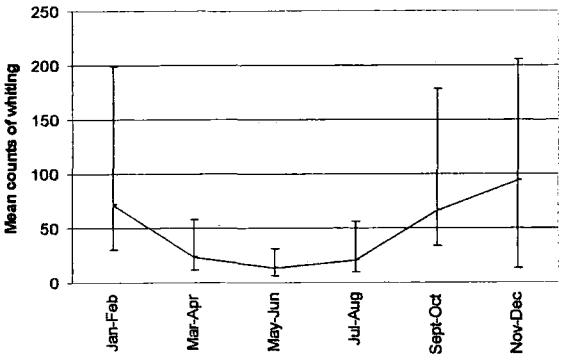


c)

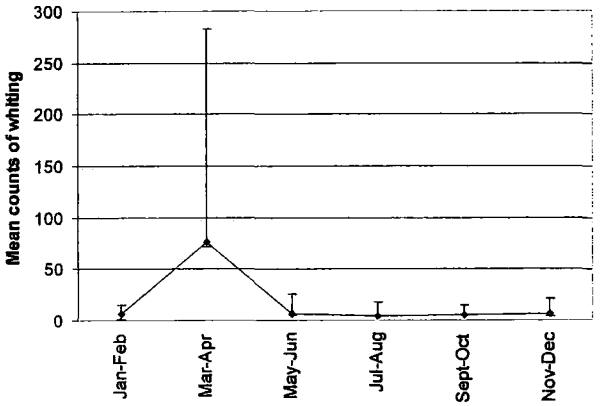
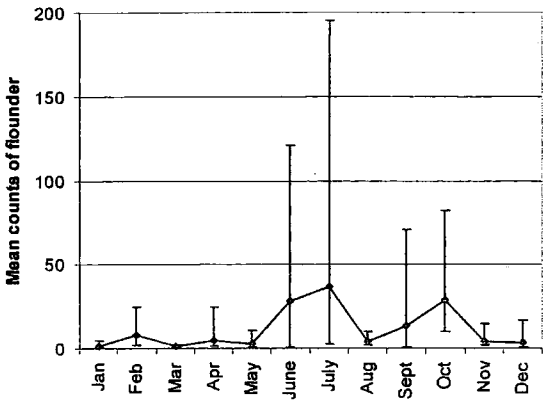
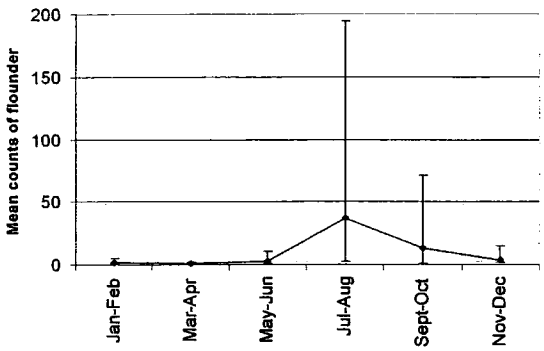


Figure 3.6. Comparison of seasonal changes in abundance (mean and range) of whiting counted in a) intake water screens of Hartlepool Power Station, Tees Estuary, 1992-2002 (Calculated from raw data provided by D. Bastreri, Environment Agency, 2002) with the seasonal number of whiting consumed by b) cormorants and c) harbour seals from the Tees Estuary

a)



b)



c)

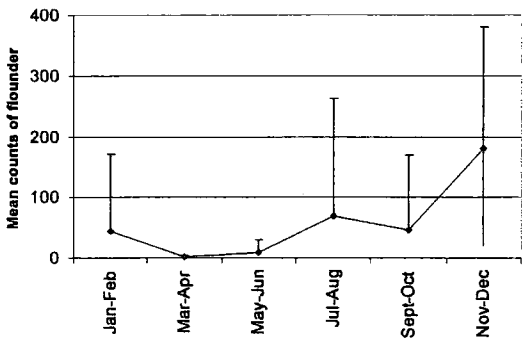


Figure 3.7. Comparison of seasonal changes in abundance (mean and range) of flounder counted in a) intake water screens of Hartlepool Power Station, Tees Estuary, 1992-2002 (Calculated from raw data provided by D. Bastreri, Environment Agency, 2002) with the seasonal number of flounder consumed by b) cormorants and c) harbour seals from the Tees Estuary

3.6 SEASONAL DIFFERENCE BETWEEN THE BIOMASS OF PREY CONSUMED BY HARBOUR SEALS AND CORMORANTS IN THE TEES ESTUARY

Biomass of species consumed seasonally was considered the more relevant method of measuring consumption in relation to intake of metals by seals and cormorants. Seasonal diversity of species consumed was determined to consider the quality of seasonal seal diet. The seasonal difference between the mean biomass of all prey consumed by harbour seals and cormorants in the Tees Estuary is shown (Figure 3.8). Harbour seals consumed a higher mean mass of total prey in January to February and May to June and a lowest mean mass of total prey in July to August. Cormorants consumed a higher mean mass of total prey in November to February and the mean mass of total prey consumed was lowest in May to October. Harbour seals consumed a higher mean mass of total prey than cormorants in January-February and May to June, whereas cormorants consumed a higher mean mass of total prey than seals in July to August.

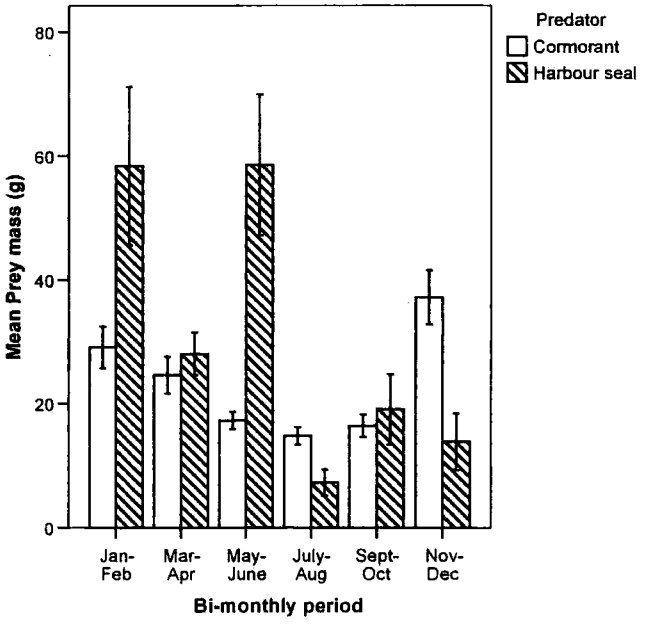


Figure 3.8. Mean prey mass consumed by seals and cormorants per bi-monthly period (error bars show 95% Confidence Intervals)

A one-way ANOVA was conducted to determine whether there was a significant difference in biomass between bi-monthly periods for each of harbour seals and cormorants. The difference between the biomass of prey consumed bi-monthly by harbour seals was statistically significant ($F = 37.5$, d.f. = 5, $p < 0.001$). The difference between the biomass of prey consumed bi-monthly by cormorants was statistically significant ($F = 43.2$, d.f. = 5, $p < 0.001$).

Post-Hoc tests were conducted to assess between which bi-monthly periods the mass of prey consumed was different (Table 3.9).

Table 3.9 a) Post-Hoc tests (LSD) to assess significant difference of mass of prey consumed by harbour seals between bi-monthly periods

	MA	MJ	JA	SO	ND
JF	***	NS	***	***	***
	MA	***	***	NS	***
		MJ	***	***	***
			JA	*	NS
				SO	NS

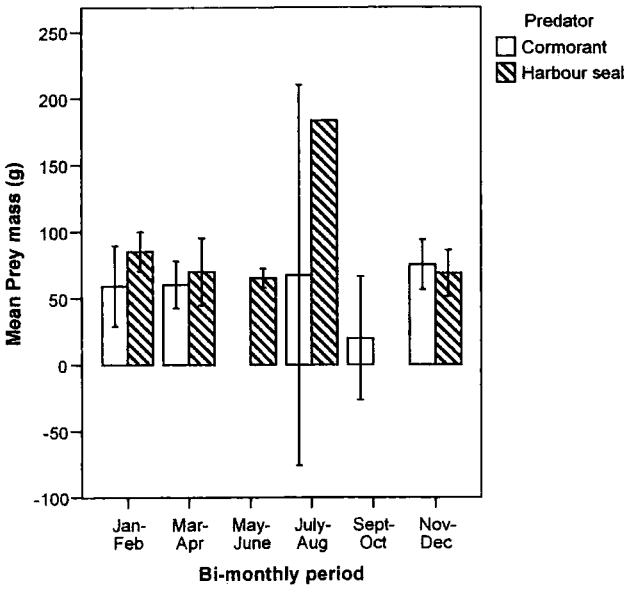
Table 3.9 b) Post-Hoc tests (LSD) to assess significant difference of mass of prey consumed by cormorants between bi-monthly periods

	MA	MJ	JA	SO	ND
JF	*	***	***	***	***
	MA	***	***	***	***
		MJ	NS	NS	***
			JA	NS	***
				SO	***

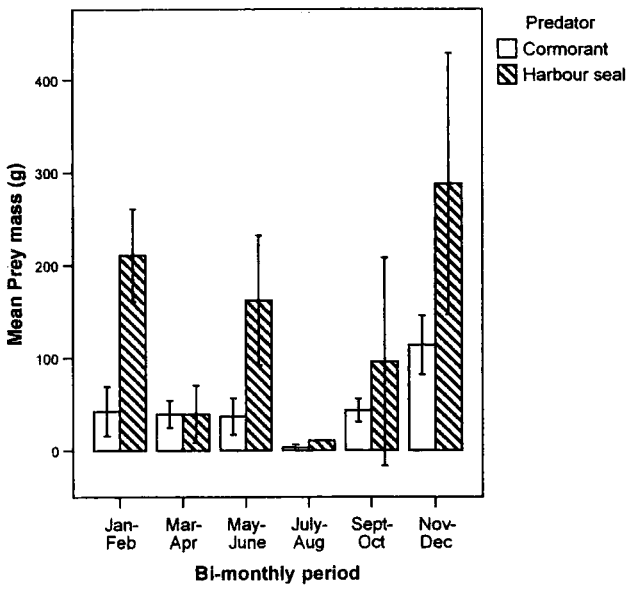
An independent t-test was conducted to determine whether there was a significant difference in biomass consumed by seals and cormorants. The difference between the biomass of total prey consumed by harbour seals and cormorants was statistically significant ($t = -3.6$, d.f. = 8112, $p < 0.001$).

The seasonal difference between the mean biomass of the main prey groups consumed by harbour seals and cormorants in the Tees Estuary is shown (Figure 3.9).

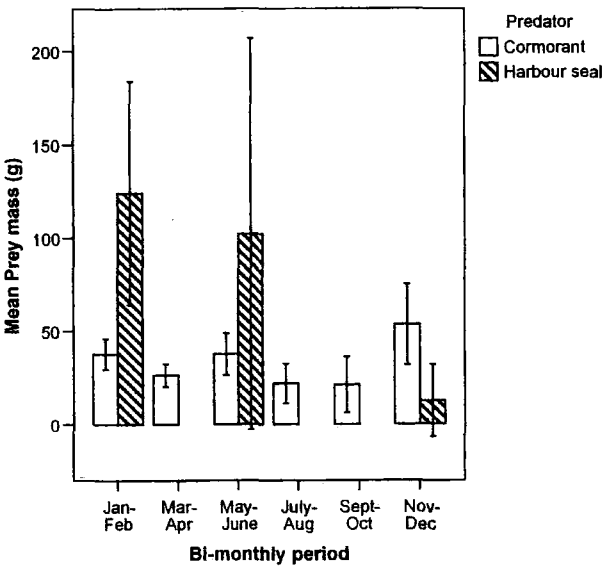
a)


Predator
Cormorant
Harbour seal
Mean Prey mass (g)
250
200
150
100
50
0
-50
-100
Jan-Feb
Mar-Apr
May-June
July-Aug
Sept-Oct
Nov-Dec
Bi-monthly period

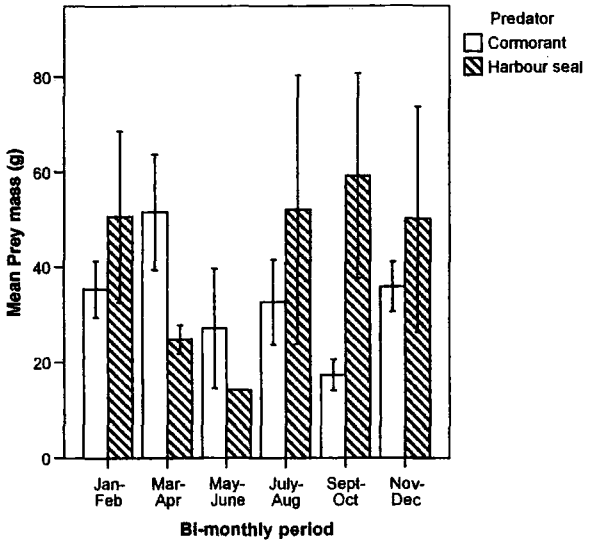
b)


Predator
Cormorant
Harbour seal
Mean Prey mass (g)
400
300
200
100
0
Jan-Feb
Mar-Apr
May-June
July-Aug
Sept-Oct
Nov-Dec
Bi-monthly period

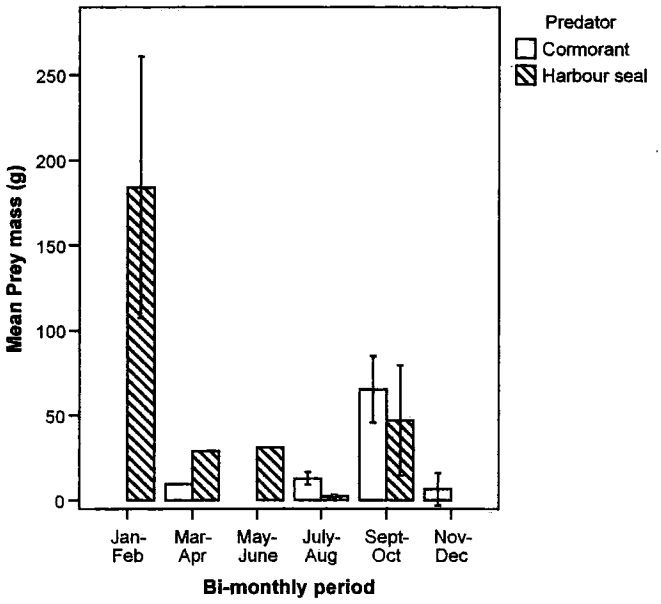
c)


Predator
Cormorant
Harbour seal
Mean Prey mass (g)
200
150
100
50
0
Jan-Feb
Mar-Apr
May-June
July-Aug
Sept-Oct
Nov-Dec
Bi-monthly period

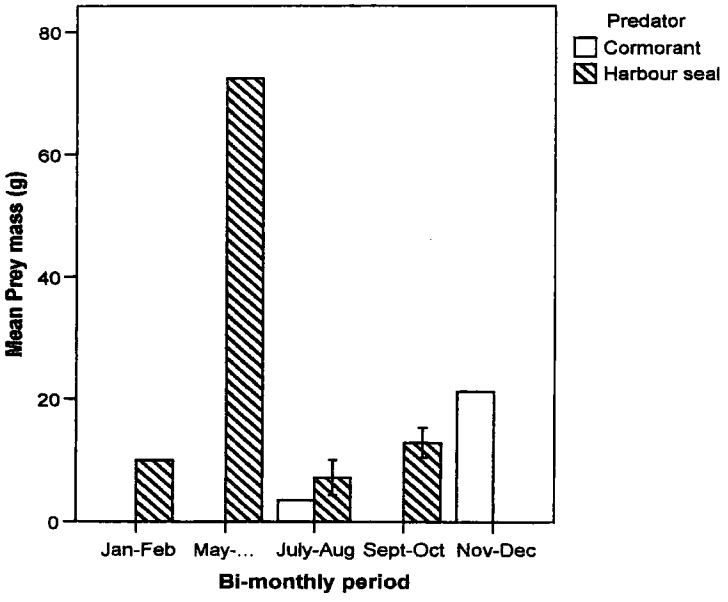
d)


Predator
Cormorant
Harbour seal
Mean Prey mass (g)
80
60
40
20
0
Jan-Feb
Mar-Apr
May-June
July-Aug
Sept-Oct
Nov-Dec
Bi-monthly period

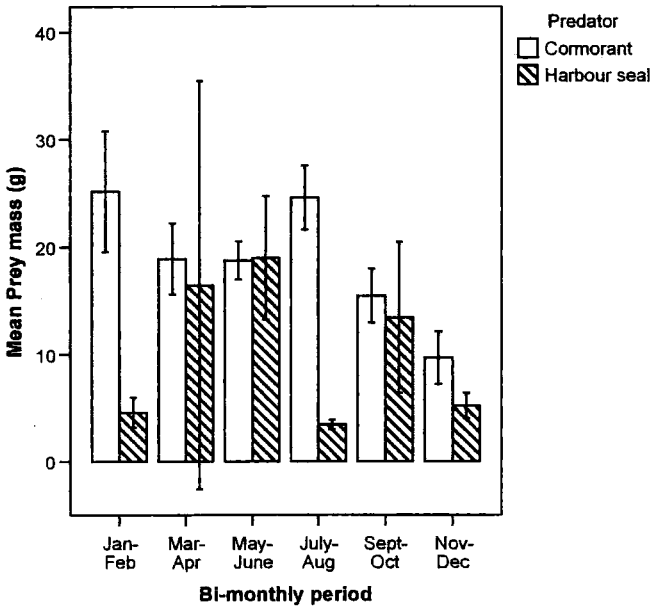
e)


Predator
Cormorant
Harbour seal
Mean Prey mass (g)
250
200
150
100
50
0
Jan-Feb
Mar-Apr
May-June
July-Aug
Sept-Oct
Nov-Dec
Bi-monthly period

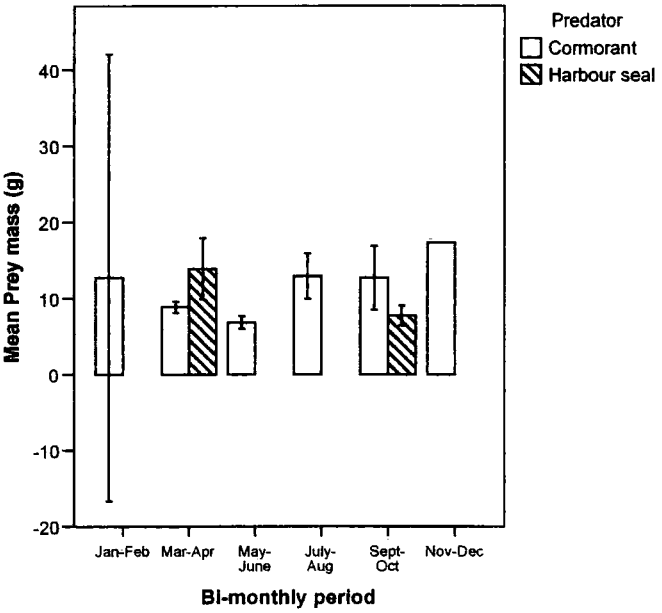
f)


Predator
Cormorant
Harbour seal
Mean Prey mass (g)
80
60
40
20
0
Jan-Feb
May-...
July-Aug
Sept-Oct
Nov-Dec
Bi-monthly period

g)


Predator
Cormorant
Harbour seal
Mean Prey mass (g)
40
30
20
10
0
Jan-Feb
Mar-Apr
May-June
July-Aug
Sept-Oct
Nov-Dec
Bi-monthly period

h)


Predator
Cormorant
Harbour seal
Mean Prey mass (g)
40
30
20
10
0
-10
-20
Jan-Feb
Mar-Apr
May-June
July-Aug
Sept-Oct
Nov-Dec
Bi-monthly period

i)

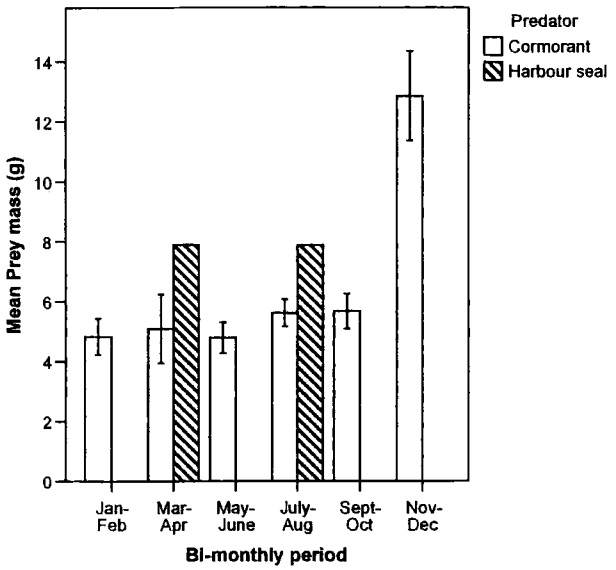


Figure 3.9. Mean mass of main prey species consumed by seals and cormorants per bi-monthly period (error bars show 95% Confidence Intervals) a) poor cod b) cod c) saithe d) whiting e) herring f) sprat g) pleuronectids h) lesser sandeel i) lesser weever

A two-way ANOVA was conducted on each of the main prey groups to determine whether there was a significant difference in biomass between predators and seasons (Table 3.10).

Table 3.10. Results of two-way ANOVA to test for difference in biomass of the main prey species consumed between predators and between bi-monthly periods

Species	Predators	Bi-monthly periods
Poor cod	$F = 7.5$, $df = 1$, $p<0.01$	$F = 2.7$, $df = 5$, $p<0.05$
Cod	$F = 18.3$, $df = 1$, $p<0.001$	$F = 13.5$, $df = 5$, $p<0.001$
Saithe	$F = 6.4$, $df = 1$, $p<0.05$	$F = 2.7$, $df = 5$, $p<0.05$
Whiting	$F = 5.3$, $df = 1$, $p<0.05$	$F = 2.9$, $df = 5$, $p<0.05$
Gadidae	$F = 92.7$, $df = 1$, $p<0.001$	$F = 27.7$, $df = 5$, $p<0.001$
Herring	$F = 0.1$, $df = 1$, *NS*	$F = 27.1$, $df = 5$, $p<0.001$
Sprat	$F = 0.3$, $df = 1$, *NS*	$F = 40.5$, $df = 5$, $p<0.001$
Clupeidae	$F = 1.2$, $df = 1$, *NS*	$F = 15.7$, $df = 5$, $p<0.001$
Pleuronectidae	$F = 12.9$, $df = 1$, $p<0.001$	$F = 8.0$, $df = 5$, $p<0.001$
Sandeel	$F = 0.1$, $df = 1$, *NS*	$F = 4.9$, $df = 5$, $p<0.001$
Weever	$F = 0.7$, $df = 1$, *NS*	$F = 27.6$, $df = 5$, $p<0.001$

3.7. DISCUSSION OF THE FORAGING BEHAVIOUR OF PREDATORS FROM THE TEES ESTUARY

Hard part analysis of harbour seal faeces and cormorant pellets in the Tees Estuary indicated that there was partial dietary overlap between the diets of the two predators. The prey profile for both predators was similar with preference for a few dominant prey species but other prey being taken less frequently. There was considerable overlap between the prey species consumed by both predators with either gadids or pleuronectids being the dominant prey consumed. The gadids, poor cod, cod and whiting were dominant in the seal diet followed by pleuronectids, whereas pleuronectids were dominant in the cormorant diet compared to gadid species. The most dominant gadid species in the cormorant diet were whiting and cod. Hall *et al* (1998) also recorded whiting and pleuronectids to be the most dominant species in the diet of harbour seals in the Wash in 1990-1992 (Hall *et al*, 1998). In the Wash, however, gadids and pleuronectids only accounted for about half the diet. A number of other species were consumed and the main prey species changed with seasonality. In the Tees Estuary gadids and pleuronectids were the predominant prey.

The diet of cormorants exhibited greater niche breadth than that of the seals. The harbour seals in the Tees Estuary consumed 15 fish species and two species of Crustacea and cormorants in the Tees Estuary consumed a total of 28 species of fish and two species of Crustacea. Thirteen of the species of fish consumed by the seals and both Crustacea species were counted in the Hartlepool Power Station intake screens, indicating that the seals mainly utilize the prey source available inshore of the Tees Estuary. The two fish species that were found in seal faeces but were not recorded in the Hartlepool Power Station intake screens were haddock and poor cod. This indicates that the seals do migrate outside of the estuary to feed but the seals would have to be radio-tracked to discern whether all the seals forage outside of the estuary or if a few individuals avoid competition by foraging further afield. Eight of the fish species consumed by the cormorants were not counted in the Hartlepool Power Station intake water screens during 1999-2002: haddock, poor cod, long rough dab, *Hippoglossoides platessoides*, wrasse and megrim, *Lepidorhombus whiffiagonis* and freshwater perch, *Perca fluviatilis* and roach, *Rutilus rutilus*. The greater niche breadth

of prey consumed by cormorants suggests that either they travel further to hunt, that seals void remains of offshore species out to sea or that the pellets more inclusively reflected the diet of cormorants. Faecal samples collected on Greatham Creek may represent only meals near the haul-out site and not those out to sea. Harbour seals and cormorants have not been observed feeding in the estuary despite regular monitoring. The feeding distribution of these predators can not therefore be determined without further investigation. The remains of two freshwater prey species in cormorant pellets indicates that at least a few, individual cormorants do migrate inland.

Studies show that harbour seals and cormorants are opportunistic feeders, adjusting their foraging patterns to take advantage of locally and seasonally abundant prey. Seasonal variation in harbour seal diet was observed in the Moray Firth (Pierce *et al*, 1991, Thompson *et al*, 1991, Tollit and Thompson, 1996, Tollit *et al*, 1997a), in Orkney (Pierce *et al*, 1990), in Shetland (Brown and Pierce, 1997; 1998), in the Wash (Hall *et al*, 1998) and in the Skaggerrak and Kattegat (Härkönen and Heide-Jørgensen, 1991). Inconsistencies with regard to which prey species are dominant in seal diet, even in seals from the same location, suggest that seals switch prey and adjust their foraging pattern to find alternative prey when food conditions change (Brown and Mate, 1983; Jeffries, 1986; Olesiuk, 1993). These may reflect, to some extent, the large inter-annual variations in recruitment rates of potential prey and predators are expected to display similar foraging behaviour. In the Tees Estuary there was evidence of seasonal changes in prey consumed. Harbour seals consumed a greater diversity of species during the summer months when gadid species were less available according to the fish counts conducted by the EA (Figure 3.6a). Seals then appear to switch to other prey, such as pleuronectids, clupeids and Crustacea. Sprat became an important prey species in the seal diet by occurrence during September to October and herring became an important prey species in the seal diet by occurrence during July to August, corresponding with the clupeids being most available during their summer migration into the Tees Estuary (Bastreri, D., Environment Agency, pers.comm.). Figures 3.4a and 3.5a show this increase in clupeid numbers and Figure 3.6a shows the increase in the main gadid species found in the Tees Estuary, the whiting, to occur during the winter

months. The highest numbers of cod in the Tees Estuary were also counted during the winter months (Bastreri, D., Environment Agency, pers.comm.). Seals may switch to consuming greater proportions of clupeids in the summer due to a reduction in gadid numbers or an increase in clupeid numbers. A number of studies have documented that harbour seals prefer clupeids and consume small gadids and lesser sandeels when clupeids are not available (Härkönen, 1988; Härkönen and Heide-Jørgensen, 1991; Thompson *et al*, 1997). Despite this preference for clupeids harbour seals in the Skaggerak-Kattegat area fed predominantly on bottom-dwelling fish living on soft bottoms above 30 m where vegetation was scarce or diminished (Härkönen, 1988). The greater mass of sprat and herring consumed by harbour seals however, was during January to February and May to June, respectively (Figure 3.9 e and f). This indicates that they are feeding on larger individuals rather than a higher frequency of small individual clupeids.

Pleuronectids were the dominant prey in the diet of cormorants from the Tees Estuary by occurrence. They consumed a greater diversity of species during the winter months. This suggests that while cormorants most frequently consumed prey is pleuronectids they also consume gadids when they are abundant in the winter months. Several researchers have found that cormorants predominately forage on benthic prey species (Leopold *et al*, 1998; Goutner *et al*, 1997). In the Dutch Waddensea pleuronectids comprised 73% of the cormorant diet by numbers and 79% of the cormorant diet by mass (Leopold *et al*, 1998). In Mediterranean estuaries 67% of cormorant diet comprised benthic species and 29% was comprised of pelagic species (Goutner *et al*, 1997). Cormorants also consumed large numbers of weever in the Tees Estuary but this species was rarely consumed by the harbour seals. Cormorants did consume some clupeids in the summer but considerably smaller numbers than were consumed by seals.

In the southern North Sea dragonet feeding activity, leading to their growth, appears to be restricted to the warmer months of May/June to October (Hall *et al*, 1998). Dragonets from the Tees Estuary were found in the harbour seal diet in March to June and the cormorant diet in May to August suggesting that they are consumed during the warmer months

because they increase their feeding activity and are therefore more available to predators. Increased consumption of dragonet in the warmer months is also seen in harbour seal diet in the Wash (Hall *et al*, 1998). Prime and Hammond, (1987; 1990) suggest that crab remains in seal faecal samples correlate with the weaning of pups. The greatest percentage of crab remains in seal diet was 22% during May to June. Harbour seals in the Tees Estuary give birth between June and July and the pups wean in August. The high percentage of crabs consumed in May to June is not consistent with them being the prey of young seals.

In the Moray Firth, harbour seal diet was either dominated by dermersal and benthic species or by pelagic species (Thompson *et al*, 1991). Changes in availability and quality of prey may cause population energy requirements to vary, so producing differences in individual activity costs, affecting body condition. In the Moray Firth, the condition index of seals was higher in the spring after an abundance of clupeids and the body lengths and mass of yearling seals was greater. Small shoaling fish such as herring, sprat and lesser sandeel provide high energy densities. Sprat and herring are the most abundant fish in the Tees Estuary, whereas only a small number of sandeel were counted in the Hartlepool Power Station intake water by the Environment Agency. A decrease in food availability may result in the use of more distant feeding grounds, leading to an increase in energy requirements. Seals in the Moray Firth were found to be anaemic after winters when gadids and lesser sandeels became the main prey, rather than clupeids. Anti-metabolite in gadids is suspected to possibly cause haematological changes (Thompson *et al*, 1997). Decreased body size and anaemia reduces the oxygen storage capacity, limits the diving ability and so reduces the prey capture facility. Between year variations in food availability and diet therefore have a number of effects at individual and population level. Juvenile survival may change, for example, either directly or due to changes in maternal investment (Costa *et al*, 1989). Female fur seals were observed to significantly increase their foraging effort during low prey availability (Boyd *et al*, 1994). A reduction in food availability may cause a decrease in the growth rate and so an increase in age of sexual maturity (Laws, 1956). These effects at an individual level may limit population size. Seasonal food availability for harbour seals in the Tees Estuary may affect their ability to reproduce successfully. Clupeids comprised

only a small proportion of the harbour seal and cormorant diet in the Tees Estuary despite being the dominant species in fish counts conducted at Hartlepool Power Station by the Environment Agency, particularly during the summer months.

Prey availability should be considered both in terms of abundance and also whether fish can locate the prey (Elliott and Hemingway, 2002). In addition, availability must be assessed in relation to the physical nature of the estuary. The visual characteristics of prey may play a role in determining whether it is eaten or rejected. Experimental studies in prey detection and acceptance in fish suggest that the important visual characteristics of prey are: contrast with the background, size, movement, shape, colour and unusual form. The maximum distance at which potential prey can be detected is the reaction distance on this depends on the size of the prey (the distance is greater for larger prey), the turbidity of the water and the resolving power of the eye of the predator. If more than one prey is present, then the prey appearing largest to the predator, as the result of size and proximity, is the one likely to be attacked. Larger gadid species are more likely to be preyed on by harbour seals and cormorants than smaller, fast swimming pelagic fish. In seasons when there are few large gadids available small pelagic fish will be difficult to detect in the turbid waters of the estuary and therefore harbour seals and cormorants in the Tees Estuary tend to select slow, easy to catch benthic fish and crabs. Benthic fish tend to have cryptic colouration and behaviour, but they can be detected by touch as well as vision.

Prey items may also be selected to provide the maximum profitability to the predator (Elliott and Hemingway, 2002). Profit is usually measured as net rate of energy gain, that is total energy gained per unit time by the forager minus the energy costs of foraging which depends on the energy content of the prey type, the time it takes to encounter a prey type and the energy costs of searching for and capturing that prey type. Clupeids may provide more calories per gramme but since they are small, fast and difficult to detect in turbid waters their profitability is low. Their profitability is increased in seasons (or locations) when they are highly abundant and so the energy costs of encountering and capturing for the prey is decreased. Small benthic fish and Crustacea provide less calories per gram but the

energy expended in encountering and capturing these prey will be relatively low so the profitability of these species as prey is relatively high. Alternatively, the seal faeces voided at Greatham Creek my contain more benthic fish, whilst pelagic fish are caught by seals further out to sea so the faeces are voided in the sea. In this case it would be expected that cormorant pellets would have a greater quantity of clupeid remains than seal faeces as they return nightly to roost and regurgitate their pellet.

The harbour seal and cormorant diet is dominated by gadids and pleuronectids so the predators do appear to feed on the most abundant, and probably the most readily caught, prey species in the geographical area (Härkönen, 1987). Prey selection is therefore likely to relate to temporal changes in abundance (Pierce *et al*, 1991; Tollit, 1996). Some studies have demonstrated individual foraging specialization in marine mammals (Hoelzel *et al*, 1989; Harwood, 1990, Tollit, 1996). Potential determinants of prey choice are variation in prey size, shape and the effect of handling and capture times (Hoyle and Keast, 1987; Sinclair *et al*, 1994; Wanzenbock, 1995), inter-specific and age related differences in prey behaviour (Dipper, 1987) or prey quality (Hislop *et al*, 1991). The range of prey species present in faecal samples may be a result of individual seals feeding on a specialist diet rather than the seals being generalist feeders. Lack of information on the source of the faeces precludes determination of whether prey choice is opportunistic or a number of specialist individuals.

Gadids accounted for 53.4% of the harbour seal annual diet by weight along the South East Shetland coastline (Brown and Pierce, 1998). The dominant gadid fish were whiting (25.3%) and saithe (11.1%). Sand eels accounted for 28.5% of the harbour seal diet by weight and pelagic fishes accounted for 13.8%. Gadids also accounted for the greater percentage (78%) of the harbour seal annual diet by weight in the Tees Estuary. Cod were the dominant gadid fish in the diet of harbour seals from the Tees Estuary (37%), followed by whiting (20%), poor cod (18%) and then saithe (3%). Sand eels only accounted for 0.2%. This was probably due to sandeel being located offshore of the Tees Estuary so either

the seals were not predating on them as they were too distant or the seals that predating on them were not defaecating on Greatham Creek.

Harbour seals and cormorants from the Tees Estuary consumed a higher biomass of prey in the winter months than the summer months. This reflects the greater number of gadids consumed during the winter months. Harbour seals consumed a greater biomass of prey in the winter months than the cormorants. This is expected as seals have a higher energetic demand but seals consumed a lower biomass of prey during the summer months. This may be due to the higher number of clupeids consumed which provide high energy densities consumed by seals than cormorants that consume a large number of 0 to 1 year old pleuronectids which have a low energy content. This may also be due to the number of clupeids being consumed by seals being under-estimated as this species have small, fragile otoliths which may be eroded more readily than otoliths from other prey species.

There was partial overlap in the diets of harbour seals and cormorants foraging in the Tees Estuary with gadids and pleuronectids being the dominant prey consumed. Cormorants preferred pleuronectids and seals preferred gadids however, and the TWINSPAN analysis shows some difference in the species and sizes of gadids preferred. Cormorants consumed considerable numbers of weever which were rare in the seal diet and seals consumed considerable numbers of clupeids and shore crab which were less frequent in the cormorant diet. Cormorants exhibited a greater niche breadth of prey, including two freshwater species. The overlap of diet suggests that some exploitative competition may be present between the two predators but they avoid direct competition by consuming different proportions and sizes of prey and cormorants consume a greater range of species, including two freshwater species which are not accessible to the seals.

CHAPTER 4. HEAVY METAL CONCENTRATIONS IN THE PREDOMINANT FISH AND CRUSTACEAN SPECIES OF THE DIET OF TOP PREDATORS IN THE TEES ESTUARY

4.1 INTRODUCTION

This chapter considers variation in concentrations of heavy metals of fish and Crustacea within the Tees Estuary, in order to provide information on metal contamination within these key trophic links and for input into seal and cormorant pollutant input /excretion budgets. The concentrations of seven heavy metals, which are of concern in the Tees Estuary due to high levels in the water or sediment, high toxicity or both, were determined in the predominant fish and crustacean species in the harbour seal, *Phoca vitulina* diet and some of the main prey species in the cormorant, *Phalacrocorax carbo* diet. The seven heavy metals were the essential metals, zinc (Zn), copper (Cu), chromium (Cr) and arsenic (As) and the non-essential metals, lead (Pb), cadmium (Cd) and mercury (Hg). Metal concentrations were investigated for species specific differences, variation within Crustacea and fish species, interaction between metals and affects of season and body size. The influence of these factors on metal concentrations is important in predicting metal intake by predators particularly in relation to species, body size and season.

4.1.1. Effects of heavy metals on estuarine fish and Crustacea

In trace amounts, some heavy metals, such as Zn, Cu and Cr are essential for the growth and normal development of estuarine biota (Furness and Rainbow, 1990). Intracellular concentrations of essential metal ions are generally maintained at optimal levels by homeostatic mechanisms. There is a 'window of essentiality' of required quantities and mechanisms have evolved to sequester, transport and utilize essential metals at levels above those required (Bryan and Langston, 1992). Kress *et al* (1999) reported that Zn and Cu levels in fish muscle tend to be relatively uniform, regardless of species and location. This indicates a substantial degree of physiological regulation. The paucity of regulation mechanisms for non-essential metals, such as Pb, Cd and Hg increases the potential for toxic effects and bioaccumulation from these elements (Bryan and Langston, 1992; Kennish, 1992).

All heavy metals can become toxic to estuarine organisms at a threshold bioavailability (Elliott and Hemingway, 2002). These threshold concentrations depend on the ambient metal concentrations and they also vary between metals, between species and with the physicochemical characteristics of the area. Responses of organisms to the presence of excessive metals in their medium or food depend on the ability of the species to regulate concentrations attained in their tissues or to detoxify and store metals in relatively harmless forms. Toxic effects of metals occur when excretory, metabolic, storage and detoxification mechanisms are no longer capable of matching uptake rates although there are few examples of chronic exposure of organisms from the top trophic levels to metal concentrations that has resulted in death. An extreme example is the death of over 800 people during the 1950s in Minimata Bay, Japan. They were poisoned by high concentrations of Hg in the water and hence, in the fish that were a major component of their diet. Sublethal affects of metal toxicity may occur, include changes in feeding behaviour, respiration, metabolism and digestive efficiency in animals and a range of pathological responses, such as tissue inflammation and degeneration, lack of repair and regeneration of damaged tissue neoplasm formation, genetic derangement and growth inhibition (Kennish, 1997). Non-essential metals, tend to have sublethal effects at lower concentrations, particularly Hg in its methylated organic form and Cd. Hg and Cd are Red List substances (based on Annexes of the 1992 Paris Convention) whose control is a priority, whereas Zn, Cu, Pb, As and Cr are grey list quantities whose discharge may be permitted in carefully controlled quantities (McLusky and Elliott, 2004).

Heavy metals tend to be more toxic and bioaccumulative in their organic form because they are more readily absorbed than inorganic forms as are lipophilic and tend to pass directly through membranes (Rainbow, 1988). Inorganic metal ions may be so insoluble that they pass through the digestive system after ingestion without toxic effects whereas toxicity can be enhanced if ions bind to organic ligands (Walker *et al*, 1996). Organo-Hg and organo-Pb are extremely toxic (Crompton, 1998).

Bioaccumulation of metals by organisms is influenced by age, sex, body mass, growth, reproductive cycle, body lipid, diet, and the estuarine environment including seasonality, salinity and temperature (Phillips, 1980; Lawrence and Hemingway, 2003). Salinity and temperature affect physiology and metabolism, which in turn affect the net uptake of trace metals (Phillips, 1980). The type, position and frequency of metal discharge to the estuary influences seasonal variation in trace metal availability (Phillips, 1980). The biota of the lower estuary would be expected to exhibit higher rates of trace metal uptake in winter due to high run-off.

The bioavailability of metals in solution has decreased in recent years in many British estuaries, including the Tees Estuary, due to reduced heavy metal discharge (Davies *et al*, 1991; Jones and Turki, 1997). The greatest quantity of heavy metals, originating from historic discharge, now tends to be contained in particulate fractions in the sediment (Dallinger *et al*, 1987). Sea-bed sediments tend to accumulate heavy metal concentrations of approximately three to five times those in the water column (Kennish, 1992). In a study of Hg contamination of an estuarine ecosystem, Elliott and Griffiths (1986) found ninety-seven per cent of the standing mass of Hg in the sediment and about one per cent in the biota of the Forth estuary. The content of heavy metals in estuarine bottom sediments is a function of their chemical and mineralogical composition related to the grain size of the particles. Most heavy metals are associated with the fine-grained fraction of the sediments as they contain substances that complex or chelate metal ions such as clay, chlorine ions and humic acid. The uptake of dissolved trace metals onto solid phases and the release of material into solution from particulate phases by dissolution, desorption and autolytic respiratory biological processes are important processes to the overall bioavailability of elements. Bioavailability also varies with the degree of element recycling. Disturbance of heavy metals bound to dead organisms, faeces and crustacean moulted exoskeletons generally account for more than 90% of the vertical transport of metals in the water column (Bryan, 1976).

Metal concentrations are expected to vary in body tissues of the biota depending on their mobility and position in the water column (Bryan, 1971; Rainbow, 1995). Bryan (1971) suggests that sessile organisms will accumulate metals more than mobile species because they are unable to avoid high concentrations. Benthic organisms are exposed to metal levels in the sediment, whereas pelagic organisms are exposed to concentrations of metals in the ambient water and these tend to be lower. Metals associated with particles may become available to benthic organisms after ingestion and burrowers may be bathed in interstitial water of the sediment or by their own irrigation currents interacting with interstitial water (Rainbow, 1995). Exposure time of biota within the estuary may also cause variable chronic toxicity with migratory species expected to have lower body burdens than residents, since metal levels tend to be higher in the estuary than offshore waters (Bryan and Langston, 1992).

Concurrent presence of heavy metals effects the uptake of metals, and hence their bioaccumulation. Interactive effects between metals vary from synergistic (where the concurrent presence of one trace metal enhances the bioaccumulation of another), to antagonistic (where the concurrent presence of one trace metal decreases the bioaccumulation of the first) (Rainbow *et al*, 2000). Most of the literature on the fate and effects of metal interactions in the aquatic environment report data obtained from single metal exposures, whereas in the field, organisms are generally exposed to mixtures of contaminants. Four elements with the potential to elicit multiple toxic effects at high levels are Hg, Cd, Pb and As (Becker, 2000). Metals may compete at active uptake sites and may result in metal deficiencies if uptake of essential metals were reduced (Walker *et al*, 1996). These interactions often involve metallothionein (MT) (Lawrence and Hemingway, 2003). MT is thought to be involved in the normal homeostatic control of intracellular Zn and Cu levels, but will also bind non-essential metals, such as Cd and Hg. Exposure to any of these metals will cause increased synthesis of MT and increased binding sites in the cells. Increased levels of MTs can have detrimental effects on cells through perturbation of essential metal metabolism.

It is probable that Zn, Cu, Pb and Cd, are in competition for binding sites as they all have divalent forms. Positive correlations between Cd and Zn concentrations in marine fish have been shown to exist suggesting these metals share one or more routes of uptake from solution (Thompson, 1990). There was a positive correlation between Cd and Pb and a negative correlation between As and Cd in the liver and muscle of saithe, *Pollachius virens* and flounder, *Platichthys flesus* (Julshamn and Grahl-Nielson, 1996). Zn and Cd in solution competed for uptake in the prawn, *Palaemonetes elegans* (Nugegoda and Rainbow, 1995) and shore crab, *Carcinus maenas* (Rainbow *et al*, 2000). Zn was taken up at a higher rate than Cd for the same total dissolved metal concentration, but at a lower rate than Cd per free metal ion concentration. Selenium can modify the toxicity of Hg, Cd and As. Cu has the potential to interact with selenium and possibly to compete with Hg for selenium. There was competition for binding sites on the surface of fish gills between the light metal, calcium and divalent metal ions which may influence metal uptake and toxicity (Pagenkopf, 1983).

Interactions of metals may change from antagonism to synergism with change of concentration, so the interaction at one concentration should not be extrapolated to other exposure levels. The rate of Zn uptake by *Palaemon elegans* decreased and the rate of Cd uptake increased in comparison with the uptake rates of each metal in single metal exposures of 20 $\mu g\ l^{-1}$. Cd appears to act antagonistically during the uptake of Zn, at least at these concentrations. Zn and Cd did not consistently interact synergistically in shore crab at 50 $\mu g\ l^{-1}$ (Rainbow *et al*, 2000). A higher percentage of total dissolved Zn and Cd present in the form of the free metal ion is bioavailable to invertebrates at lower salinities (Furness and Rainbow, 1990).

Pollutant concentrations in biota tend to change seasonally. Phillips (1980) suggested that any of three primary factors may contribute to the seasonality of pollutant levels in an aquatic environment, either individually or in combination: delivery of the pollutant to the estuary (and dilution of pollutant by receiving waters), the physiology of the organism, particularly the sexual cycle and changes in body mass and changes of ambient water

characteristics, such as temperature, salinity and pH. Changes in body mass have a significant effect on seasonal pollutant levels. Body mass will be at its lowest after reproduction without a loss of metal content so seasonal maxima of pollutants in tissues may occur (Lawrence and Hemingway, 2003). Pollutant uptake rates are expected to increase as salinity decreases depending on the osmoregulatory intakes of dissolved metals. Increases in temperature may cause an increase in the rate of accumulation depending on the affects of physiology.

4.1.2. Regulation and tolerance of heavy metal concentrations in crustacean and fish species

Quantities of heavy metals exceeding metabolic requirements and overloading of the assimilative capacity of the system may cause toxic effects (Walker *et al*, 1996). Enzyme activating metals for example, may become enzyme inhibitors at excessively high concentrations. Some marine organisms have strategies for regulating or tolerating excessive quantities of metals (Rainbow, 1995). Pollutant load budgets are strongly influenced by the strategy of the species being studied or their prey species.

Accumulators store excessive concentrations of metals in a non-toxic, non-available form, whereas regulators maintain an approximately constant body metal concentration over a wide range of ambient metal bioavailability, indicating that metal excretion is equal to uptake. Metal concentrations in regulators are likely to reflect metabolic requirements. The degree of accumulation depends on the net difference between the rate of metal uptake and excretion (net metal content) (Dallinger and Rainbow, 1993) and dilution from body growth (Rainbow, 1990). Eighteen species of decapods regulated body concentration levels of the essential metals, Zn and Cu, to within a narrow range, whilst exposed to a wide range of ambient concentrations (Bryan, 1968). Mechanisms to regulate non-essential metals tend to be less well developed compared to essential metals (Evans and Moon, 1981). Pb bioaccumulates in the prawn, *P.elegans* and the common shrimp, *Crangon crangon* (Rainbow, 1988). Decapod species reported to bioaccumulate Cd in their tissues include

shore crab, common shrimp and the prawns, *P. elegans*, *Palaemon serratus*, and *P. montagui* (Rainbow, 1998).

The efficiency and capacity of the detoxification, metabolism and excretion mechanisms evolved to regulate heavy metals vary considerably with species and the metal load depends on the nature and extent of these metabolic processes of metal detoxification. Metal detoxification removes metals from metabolic access to vital cellular components by 'hiding' active metal ions in a metabolically inert chemical form within a protein such as metallothioneins (MTs) (Lawrence and Hemingway, 2003). Alternatively, metals can be sequestered in an insoluble form such as lysosomes, calcium granules and specialized cells (Walker *et al*, 1996). Marine invertebrates can sequester metals in specialized cells, including hemocytes and connective tissue/pore cells. Metals can also be stored in hard parts such as the exoskeleton of the Crustacea or the jaws of Nereids. Shore crabs have granules to immobolize Pb and a high concentration of haemocyanin in the blood that stores Zn and rapidly transfers accumulated Cd. This provides the organism with the potential to release detoxified metals at intervals into the gut lumen and then to be excreted in the faeces (Burgos and Rainbow, 1998). There may be preferential accumulation of a relatively non-toxic form, such as As as arsenobetaine.

MTs play an important role in the uptake and release of metals in many marine organisms including fish, aquatic invertebrates and marine mammals (Law, 1995). MTs are low molecular weight, metal-binding proteins which act as a detoxifying agent by binding excess metals to the cytosol (George *et al,* 1992). MT is synthesized as a result of exposure to metals such as Zn, Cu, Cd and Hg, thus reducing the accessibility of free metal ions (Lawrence and Hemingway, 2003). MTs were induced by waterborne Zn exposure in flatfish (George *et al*, 1992). Metal binding proteins only have a finite capacity for metal regulation. Above a certain concentration toxic effects manifest. MT binding Cd, Cu and Zn in the shore crab and edible crab, *Cancer pagarus* had the strongest affinity for Cu, followed by Cd (Rainbow, 1988). Pb will bind to MT but also has an affinity for other metabolic ligands. The prawn, *Penaeus monodon*, can detoxify metals, such as Cu and Pb,

by granule formation and excretion (Vogt and Quinitio, 1994). The protective role of lysosomes can be reversed once to storage capacity of these organelles is overloaded (Lawrence and Hemingway, 2003). The lysosomal membrane may become severely damaged resulting in severe metabolic disorders and pathological alterations. Metals can be excreted by marine organisms via a variety of routes including passive desorption, defecation, through permeable surfaces, such as the gills and by releasing detoxified granules into the lumen of the alimentary tract (Rainbow, 1990). Crustacea can also lose metals through cast molts of crustaceans.

4.1.3. Heavy metal uptake routes in Crustacea and fish

The level of contaminants in an organism is the net result of the behaviour of that material in the environment and in the organism (including uptake, storage, sequestration and excretion) and of the routes of uptake and levels in the prey (Lawrence and Hemingway, 2003). Metal concentrations at any trophic level result from a combination of uptake from water and uptake from sediment and diet (Chen *et al*, 2000). The order of priority varies with metal and taxa species. The physicochemical parameters of the aquatic medium also influence the uptake process (Dallinger and Rainbow, 1993) and ingestion of suspended particles (Rainbow, 1990). The hydrophobic nature of metal ions allows uptake from the water to occur by passive diffusion across gradients due to surface adsorption and binding to surface cells and body fluids. Metals may be taken up directly across the body surface, particularly in small and/or soft-bodied invertebrates, or at sites of high permeability, such as the gills and the alimentary tract during drinking or food ingestion.

The metal concentrations measured in pelagic organisms and in benthic organisms are expected to be influenced by the medium surrounding them. Edible crabs and flounder from an uncontaminated site were placed in tanks containing sediment taken from a site of high heavy metal contamination (Berge and Brevik, 1996). There was a clear increase of Cr and Pb levels but no increase of Cu, Cd and As levels in crab tissue after 3 months. There was only a slight increase in Zn and Cu concentration in flounder after 3 months. In this short-term experiment exposure to contaminated sediment did not necessarily result in

accumulation of considerable heavy metals in benthic crab and flounder. Pb levels in benthic fish may be relatively high as Pb has a strong affinity with the sediment and is sparingly soluble in seawater (Bryan and Langston, 1992). Methyl-Hg concentrations were higher in benthic species in contact with contaminated sediments than in pelagic fish feeding on plankton with low methylHg content (Bryan and Langston, 1992).

Bioaccumulation from diet is the predominant uptake mechanism for higher organisms with impermeable surfaces, including fish and Crustacea (Bryan, 1971). Metal concentrations taken up from the diet depend on the food source. There is a relationship between fish and macrocrustacea and the sedimentary regime in the estuary, where either the predominant food is from the infauna/ epifauna or the fish are in physical contact with the sediments (Elliott and Hemingway, 2002). Sediments have an affinity for metals so these relationships provide a readily available mechanism for uptake (Elliott and Griffiths, 1986). Metal uptake by carnivores is expected to be higher if it feeds on benthic organisms rather than pelagic herbivores (Phillips, 1980). Suspension feeders take up metals both directly from seawater and from the suspended particles collected during feeding. Deposit feeders ingest the bioavailable fractions of the sediment particles which are sinks for metals so these benthic organisms would be expected to accumulate high metal levels (Heath, 1993).

Uptake from solution was expected to be slow for Crustacea due to most of the body being covered by an impermeable cuticle and permeability being restricted to the gills (Rainbow, 1998). Zn uptake in *P. elegans* appears to be determined by physicochemical control since it correlates with the concentration of free metal ions in the exposure medium (Nugegoda and Rainbow, 1995). In contrast, food was considered to be the more important pathway for Zn accumulation in shore crab (Eisler, 1981). Chan (1990) calculated Zn uptake from food to be similar to the uptake from solution in the shore crab. The uptake rate of Cr and Pb in tissues of the edible crab increased when living in contaminated sediments but there was no increase in As, Cd and Cu (Berge and Brevik, 1996). For Cd and As this was in accordance with studies by Davies *et al* (1981) and Andersen and Depledge (1994), who found the dominant uptake route for these metals in edible crabs and shore crabs, respectively, to be

through the diet. Transfer of Cd to the grass shrimp, *Palaemonetes vulgaris*, however, was less efficient via the diet than directly from seawater (Nimmo *et al*, 1977).

In fish, the body surface is generally assumed to be impervious to the uptake of significant levels of metals from the surrounding water (Dallinger *et al*, 1987). The gills and the gut are both important pathways for metal uptake in fish but there is a lack of consensus regarding the relative importance of these two pathways. Huckle and Millburn (1990) regarded uptake from water to be the predominate uptake method for toxins in fish and so metal concentrations in fish body tissues would be expected to reflect concentrations in the surrounding estuarine environment. Aqueous Zn and Cd concentrations were good predictors of levels in lake fish from the north-eastern United States (Chen *et al*, 2000). This may be a result of Zn and Cd being transition metals with divalent cations, for which uptake is thought to be proportional to the free ion concentration. Zn and Cd differ from Hg in being more organically complexed in water than inorganically complexed, which may affect their trophic transfer but bioaccumulation may vary between Zn and Cd because Zn is regulated in tissues and Cd is not well regulated.

In contrast a number of studies have regarded food to be the dominant uptake route for metals. Hoss (1964) suggested that food is probably a more important source of Zn than seawater in the flounder because they consume a large proportion of food in relation to body size. Flounder, particularly immature flounder, tend to feed on ragworm, *Nereis diversicolor* and edible mussel, *Mytilus edulis* which can become tolerant to metal levels and accumulate high concentrations, although not necessarily in a bioavailable form. Flounder also take up considerable quantities of sediment whilst feeding and hence sediment is also an uptake route for metals (Elliott, M., University of Hull, *pers* comm.). Direct accumulation from water was thought to play a minor role relative to food in the metabolism of Zn in plaice, *Pleuronectes flesus* (Pentreath, 1973, 1976) and other species (Renfro *et al*, 1975). Zn concentrations increased significantly in the grunt, *Terapon jarbua*, in response to feeding on highly contaminated food (Zhang and Wang, 2005). In contrast, Zn concentrations increased only slightly at even the highest waterborne Zn exposure

treatment. The body burden of Zn was elevated after both waterborne and dietary exposures to Zn in the black sea bream, *Acanthopagrus schlegeli.*

Diet is considered an important pathway for Cd and Pb assimilation in marine fish (Eisler, 1981). Relatively high Cd levels in fish were attributed to a high incidence of crustaceans in the diet (Hardisty *et al*, 1974b) and not necessarily high concentrations in the surrounding environment. Swaileh and Adelung (1995) found that Cd biomagnification in the cod, *Gadus morhua* and flounder consuming the crustacean, *Diastylis rathkei* was not significant but the Cd concentration in the liver of the dab, *Limanda limanda* was 10 times higher than that in the body tissues of *D. rathkei.*

Different concentrations of As in fish species are likely to be, at least partly, a consequence of diet supporting the view that the main route of As into higher trophic levels is via the diet (Falconer *et al*, 1983). Arsenic levels in Scottish flatfish, feeding on benthic prey such as echinoderms, crustaceans, polychaetes and molluscs, tended to be higher than in the roundfish and plankton feeders. Scottish roundfish, such as whiting, *Merlangius merlangus* and saithe, feeding primarily on relatively small fish had consistently low As concentrations. Copepods also contain relatively low As concentrations and may explain the low As concentrations in their predators, such as herring, *Clupea harengus* and mackerel, *Scomber scombrus*. Haddock, *Melanogrammus aeglefinus* occasionally had higher As concentrations than whiting. This may result from haddock consuming relatively more crustaceans, molluscs and echinoderms than the whiting. Arsenic concentrations in cod tended to be relatively high despite their being predominately fish eaters, probably because they also predate heavily on crustaceans, particularly during the winter months. Pentreath (1977) emphasized the relative unimportance of sea water as a source of As in accumulation studies with plaice.

Mercury concentrations in lake fish are strongly influenced by the food web structure, indicating that plankton (via diet) is a crucial determinant of Hg burdens in pelagic fish (Chen *et al*, 2000). The importance of food type in metal accumulation is demonstrated in

the plaice, where 80 to 93% of methylHg was retained from a diet of ragworms, whereas only 4 to 42% was retained from a diet of mussels (Pentreath, 1976). Elliott and Griffiths (1986) studied Hg contamination in all the major components of the estuarine system. The highest biota concentration factors were in the wading birds, mussels and estuarine fish. These concentration factors, taken together with those for suspended material and sediments, suggest that two pathways appear to be the most critical: suspended solids to mussels to oystercatchers and sediment to infauna to estuarine dermersal fish and waders. This indicated that in the Forth estuary the top consumers most at risk from Hg contamination were resident (flounder, eelpout) rather than migratory fish (sprat, herring) or waders with a large food intake while over wintering.

Habitat preferences of prey or specialized food requirements of predators will influence metal uptake (Dallinger *et al*, 1987). Ingestion of sediment and sediment dwelling prey may be an important source of metal uptake. Fish feeding on a specialized diet of highly contaminated prey will take up more metals than generalists or specialists feeding on less contaminated prey. Elimination of susceptible species will cause metal tolerant food organisms to become dominant thus reducing species diversity and increasing the potential for biomagnification (amplification of contamination along the food chain).

4.1.4. Effects of individual heavy metals on estuarine biota

4.1.4.1. Zinc

Zinc is an essential metal for growth and normal development (Clark, 1997). More than 200 Zn-based enzymes or other proteins have been identified, across all phyla. Zn toxicity is related to the inhibition of enzymatic reactions. Excessive Zn concentrations interfere with calcium uptake and metabolism (Zhang and Wang, 2005). In fish, Zn can obstruct the gills, blocking breathing movement, as well as delaying growth and maturation (De Souza Lima *et al*, 2002).

As Zn is an essential metal organisms tend to have evolved mechanisms to cope with excess concentrations. Decapods regulate body Zn concentrations at 50-120 mg kg^{-1} dry

mass (Rainbow, 1988). There is a limit, however, above which the external concentration rate exceeds the maximum excretion rate and net accumulation occurs. Mortality occurs in *P. elegans* and shore crab when body Zn concentrations increase to double the normal, regulated levels at greater than 200 mg kg^{-1} dry mass (Rainbow, 1985; Dallinger and Rainbow, 1993). This implies that a significant proportion of absorbed Zn in decapods remains in a metabolically available form and there is little opportunity for detoxification.

Regulation is achieved by either increased excretion, reduced uptake or a combination of each of these methods. In *P. elegans* regulation is mainly achieved by increasing the Zn excretion rate above the rate of Zn uptake (White and Rainbow, 1984a). The rate of exchange (uptake and loss) of Zn in *P. elegans* was found to equal 12.9% of the total body Zn load day^{-1} at 100 μg l^{-1} at 10°C. Conversely, the rate of Zn uptake in shore crab is very low (Chan, 1990). The low uptake rate produces a low net accumulation and therefore relatively constant Zn concentrations. The low Zn uptake rate may be the result of inherent low permeability of body surfaces to electrolyte ions, possibly facilitating existence in hypersaline waters. Laboratory studies of Zn concentrations in nine species of portunid crabs fell in the range of 32.7 – 92.1 mg kg^{-1} dry mass (Chan, 1990).

Highest Zn concentrations were detected in fish associated with substrata, or those that fed on it, such as flounder (De Souza Lima *et al*, 2002). The black sea bream and the grunt were able to regulate Zn accumulation (Zhang and Wang, 2005). Zn concentrations in teleosts range from 6 - 400 mg kg^{-1} dry mass (Eisler, 1981).

4.1.4.2. Copper

Copper is an essential metal, necessary for growth and metamorphosis in many organisms (Clark, 1997). Young animals and neonates are therefore normally richer in Cu than adults. It is present in more than a dozen enzymes, with roles ranging from the utilization of iron to the pigmentation of skin. Cu is incorporated into blood pigments and is necessary for normal function of cytochrome oxidase. Decapod crustaceans need Cu to synthesize the blood pigment, hemocyanin (Dallinger and Rainbow, 1993). Biochemical functions

requiring Cu include mitochondrial activity, collagen metabolism and melanin formation (Lewis and Cave, 1982). In high concentrations Cu is one of the most toxic of the essential metals due to the same mechanisms and properties which make it an essential constituent of many metalloproteins, its ability to enter into strong complexes with organic ligands (Clark, 1997). Cu may react with proteins to denature them, so reducing enzyme activity and destroying or distorting protein structure. It can cause a number of behavioural, histological and physiological anomalies (Lewis and Cave, 1982). There is evidence of liver damage in flounder exposed to high Cu levels. In winter flounder, *Pseudopleuronectes americanus* moderate to high levels of Cu resulted in fatty metamorphosis of the liver, kidney necrosis, obstruction of the haematopoetic tissue and gross changes in the gill architecture (Lewis and Cave, 1982). Cu was also shown to affect the central nervous system and kidney functions.

Species vary greatly in their tolerance of high levels of Cu in the diet. Decapod species regulate Cu (Furness and Rainbow, 1990). Cu rich granules have been found in some crustaceans including common shrimp and the prawn, *P. elegans* and *Penaeus monodon* (Rainbow, 1998). In *P. elegans* the net Cu accumulation can reach 700 mg kg^{-1} before death, much higher than regulated levels, indicating that excess Cu is stored in a detoxified form (White and Rainbow, 1982). In laboratory studies of nine portunid crab species, Cu concentrations fell in the range of 64.1 – 128 mg kg^{-1} dry mass (Chan, 1990).

Flounder feeding in the creek of the River Fal consuming mainly ragworm (Cu accumulators) appeared able to limit Cu assimilation in the gut and the body burden did not increase (Clark, 1997). Koeller and Parsons (1977) studied the potential for Cu biomagnification in marine food chains. Cu concentrations did not increase with trophic level, but rather the species composition in the lower portion of the food chain changed and this adversely affected the higher trophic levels by decreasing food availability and diversity.

4.1.4.3. Lead

Lead does not provide beneficial or nutritional effects to organisms (De Souza Lima *et al*, 2002). It has adverse biochemical effects, many involving the inhibition of enzyme systems, such as the cytochrome P-450-linked mixed-function oxidase system. High Pb concentrations damage the central nervous system, particularly during growth (Mormede, 2001). At lower concentrations Pb can result in a variety of sublethal responses, including anaemia, depressed growth, diminished egg hatching success, fin degeneration and behavioural abnormalities (Clark, 1997). Young animals tend to absorb a greater amount of Pb than adults.

Lead levels in fish from the North Sea have decreased since the early 1980's, corresponding with the decline of Pb additive in petroleum and reduced atmospheric concentrations (Jorgensen and Pedersen, 1994). Pb concentrations in whole fish tend to be higher than in muscle and liver, possibly due to high concentrations in hard tissues, such as bone (Mormede, 2001). Inorganic Pb is generally less toxic than organo-Pb (Pain, 1995).

4.1.4.4. Cadmium

Cadmium is not an essential element, it is difficult to excrete once ingested and it is highly toxic (De Souza Lima *et al*, 2002). Even subacute Cd levels can result in physiological dysfunction in fish. It causes disruption to ionic control and calcium metabolism (Mormede, 2001). The adverse effect of Cd on juvenile plaice was depressed growth (Westernhagen *et al*, 1980). Cd is persistent, with a tendency to bioaccumulate with age and to biomagnify (Dietz *et al*, 1996). It tends to bioaccumulate at a fast rate because it is assimilated rapidly and excreted slowly (Walker *et al*, 1996). It can accumulate within invertebrates to several orders of magnitude greater than aqueous Cd concentrations (Devineau and Amiard-Triquet, 1985). It concentrates in the viscera of vertebrates, especially the liver and kidneys. Cadmium levels in fish tend to be low, often below detection limits reflecting low environmental levels (Thompson, 1990).

4.1.4.5. Arsenic

Adverse effects on estuarine and marine organisms have been reported at As levels of 100μg l^{-1} and above (Clark, 1997). The toxicity of As varies with the valency of the element. The dominant form of As in marine and brackish waters is arsenate (Francesconi and Edmonds, 1997). The more toxic and potentially carcinogenic arsenite rarely accounts for more than 20% of As in seawater. Marine algae, the base of the food chain, can accumulate arsenate from the seawater, reduce it to arsenite and then oxidize the arsenite to a large number of organo-As compounds, often to levels 1000 – 50000 times higher than in the ambient seawater (Francesconi and Edmonds, 1997, Whalley *et al*, 1999). Lower trophic marine animals then transform these arsenosugars into arsenobetaine. Arsenobetaine is the predominant form of As in marine organisms. It is pentavalent, very stable, metabolically inert and non-toxic (Becker, 2000).

Arsenic concentrations tend to be higher in marine organisms than in terrestrial organisms (Kubota *et al*, 2001). Marine fish and marine invertebrates (especially crustaceans) frequently contain concentrations exceeding 100 mg kg^{-1} dry mass of As (Phillips, 1990). Diet is considered the main source of As to marine food chains but ingested As is lost more rapidly than As absorbed from solution so the potential for As biomagnification may be low because much of the ingested As is excreted. A number of marine organisms, including some algae, crustaceans and fish, bioaccumulate As in their body tissues but it does not biomagnify (Staveland *et al*, 1993). Concentrations in organisms from lower trophic levels, such as algae and crustaceans tend to be higher than those in fish, although variability is large for each taxon. Other researchers suggest that As may biomagnify due to its high affinity to organic substances (Lawrence and Hemingway, 2003).

In a survey of As in tissues of Scottish shellfish concentrations ranged from 0.4 to 38.2 mg kg^{-1} wet mass and As concentrations in benthic organisms such as flatfish and crustaceans were relatively high compared to concentrations in roundfish and plankton feeders (Falconer *et al*, 1983). Muscle As levels of over 100 mg kg^{-1} dry mass have been reported

in a range of marine fish but other studies As rarely exceeds 20 mg kg^{-1} on either a dry or wet mass basis (Thompson, 1990).

4.1.4.6. Chromium

Chromium in biological materials is usually found in the trivalent form ($Cr^{(III)}$), but the main species of Cr in sea water is hexavalent Cr ($Cr^{(VI)}$) (Eisler, 1981). $Cr^{(III)}$ is an essential element to vital processes linked to insulin function, while Cr $^{(IV)}$ is highly toxic, with carcinogenic and ulcerative characteristics (De Souza Lima *et al*, 2002). $Cr^{(IV)}$ has a higher oxidizing potential than $Cr^{(III)}$ and easier penetration of biological membranes.

Cr is not readily bioaccumulated and has low potential to biomagnify, despite an affinity for lipids. In experiments conducted by De Souza Loma *et al* (2002) on Cr uptake in freshwater organisms inhabiting the Columbia River, concentrations were greater in lower trophic levels (algae, sponges, insect larvae and snails) than in higher trophic levels (fish and crayfish). Chromium concentrations in marine fish are generally less than 1 mg kg^{-1} wet mass and often approach the limits of detection, although higher mean Cr concentrations of 3.8 and 6.4 mg kg^{-1} wet mass were recorded in plaice and herring respectively from the Irish Sea (Murray and Portman, 1984).

4.1.4.7. Mercury

Methylmercury is a non essential metal and acts as a nerve toxin although concentrations in the environment are rarely high enough to cause death. One of the few examples is the death of over 800 people during the 1950s in Minimata Bay, Japan. They were poisoned by high concentrations of Hg in the water and hence, in the fish that were a major component of their diet. In fish it damages gills, disrupts gut absorption and chemoreception (Mormede, 2001). Hg released from natural and anthropogenic sources are primarily in the inorganic form. Micro-organisms in the intestines methylate this inorganic form to the more toxic form, methylHg (Eisler, 1981). The Hg is bound to organic ligands and becomes more soluble and hence more toxic. Approximately 40 to 90% of total Hg in the muscle tissue of shellfish and 90% of total Hg in fish and marine mammal muscle tissue tends to be

methylHg (Bryan, 1976). The bioconcentration factor in fish for methylHg is higher (10^6 to 10^7) than for non-methylHg ($<10^4$). Fish, sea-birds and marine mammals may accumulate high concentrations of Hg without detrimental effect due to low proportions of methylHg in the liver (Phillips, 1980). This is indicative of the capacity of some higher vertebrates for demethylation.

It has been suggested that Hg may biomagnify through the natural marine food chain with comparably low concentrations for lower trophic levels and marked Hg amplification for higher trophic levels including teleosts, particularly large, predatory fish, fish-eating birds and mammals (Phillips, 1980). MethylHg biomagnification is attributed to its high lipid solubility, its easy transfer across membranes and its long biological half-life. MethylHg elimination is exceptionally slow. In addition, top predators have increased longevity. As methylHg biomagnifies, concentrations can increase by several orders of magnitude up the food chain (Bryan and Langston, 1992; Dietz *et al*, 1996). Hg levels tend to be higher in adult tuna and other carnivores than in young fish within a shorter food chain (Eisler, 1981). This indicates associations between predatory behaviour, longevity and Hg accumulation. In a study of Hg contamination in all the major components of the Forth estuary, Elliott and Griffiths (1986) indicated that biomagnification of Hg across all trophic levels has not been shown. There was an increase however, along direct consumer routes in contaminated areas. That is where the consumer is a true estuarine resident or largely dependent on a single food source. Uptake of Hg through the food chain results in a significant burden in fish, although Hg concentrations were generally less than 1 mg kg^{-1} wet mass in four fish species (Kress *et al*, 1999).

4.2 MATERIALS AND METHODS FOR ANALYSIS OF METAL CONCENTRATIONS IN CRUSTACEA AND FISH

4.2.1. Sample collection

Fish and Crustacea were collected from Hartlepool Power Station cooling water intake for metal analysis. The crustacean species collected were common prawn *Palaemon serratus*, common shrimp, shore crab and swimming crab, *Liocarcinus depurator*. The fish species collected were whiting, cod, saithe, flounder, plaice, sprat, herring, lesser weever, *Trachinus vipera* and lesser sandeel, *Ammodytes tobianus*. All fresh specimens of as wide a range of sizes that were available during each visit were collected (Table 4.1). The aim was to collect a range of sizes for each species that would be representative of the individuals seasonally available in the estuary for the seals and cormorants to predate on. Collections were made bimonthly between June 1999 and December 2002. Species, date of collection, total length and wet mass were recorded. Fish were measured from the tip of the snout to the tip of the caudal fin. Shrimps and prawns were measured from the tip of the rostrum to the end of the telson and crabs were measured across the width of the carapace. Otoliths were dissected from the fish to provide reference material for dietary analysis (Chapter 2). They were then divided by species and size into polythene bags, labelled with the date and species and frozen.

Table 4.1. Median and range for the dry mass of species collected bi-monthly from the Hartlepool Power Station intake water, June 1999-December 2002

Species	Number of prey collected	Median dry mass (g)	Minimum dry mass (g)	Maximum dry mass (g)
Common shrimp	182	1.2	0.5	2.5
Common prawn	8	1.2	0.3	3.5
Shore crab	129	8.2	0.4	18.6
Swimming crab	36	3.0	0.9	10.9
Whiting	262	3.6	0.8	46.6
Cod	39	2.8	0.5	58.2
Saithe	35	6.1	0.8	13.1
Flounder	248	4.8	0.4	114.1
Plaice	36	2.4	0.6	58.2
Sprat	247	1.9	0.5	8.8
Herring	156	3.3	0.7	18.8
Lesser weever	68	3.0	1.2	8.4
Lesser sandeel	41	3.5	2.1	8.3

Avian and mammalian piscivores tend to consume whole fish, although the head of large fish may be discarded. Fish whole body metal content was, therefore, analysed for metals to represent the total body burden ingested. The exoskeleton of Crustacea may or may not be consumed but it is unlikely that it is significantly digested. Crustacea were therefore sampled both whole and separated into body parts. Twenty whole body samples of 20 common shrimp were sampled bimonthly for metal concentrations. Shore crabs and swimming crabs were analysed bimonthly for metal concentrations. Whole body metal content was analysed for 129 shore crabs and all 38 swimming crabs.

4.2.2. Sample preparation

Samples were defrosted and cut up using a scalpel. A number of measures were taken to avoid contamination: the scalpel blade was cleaned after each dissection with double distilled water and changed after every five dissections, a dissection board was used and covered with clean paper that was discarded after each dissection and the dissection board was cleaned after each dissection with double distilled water. Each sample was placed on an individual, fresh petri dish and dried in an oven at 60°C until constant weight.

The samples were taken out of the oven and dried mass per sample was recorded. The dried sample was ground to a powder of -63 μm using a pestle and mortar. The pestle and mortar were rinsed with double distilled water then trace metal analysis hydrochloric acid between each sample. This grinding up to a fine powder ensured that each sample was homogenised and provided an unbiased whole body concentration measurement, particularly in cases where subsamples of specimens were removed for replicate analysis.

4.2.3. Analysis of zinc, copper, lead, cadmium, arsenic and chromium

4.2.3.1. Digestion techniques

Small errors are possible during containment in and delivery from flasks and pipettes, weighing operations, dilution operations and reading volumes in burettes. Systematic errors were also possible such as reagent impurities or sample contamination. Care was therefore

taken when handling samples and equipment, acid-washing of glassware and covering samples to prevent contamination from dust and other sources.

Approximately 2 g of the finely ground sample was placed in an acid washed 100 ml conical flask and the mass noted. The samples were digested to obtain a solution which could be analysed for heavy metals. The sample was oxidised to break down organic matter by wet digestion. Wet digestion was used rather than dry ashing because retention losses, between the desired element and the apparatus, and volatility losses are less likely, due to lower temperatures. Approximately 2 g of sample and 10 ml of cold concentrated trace metal analysis nitric acid was added to each conical flask and a glass funnel was placed in the top. The samples were left overnight in a fume cupboard and then warmed gently on a hot plate until the material was fully oxidised (no more thick brown fumes are produced). The funnel was then removed and the heat gently increased to evaporate off all the nitric acid, leaving a solid residue. The residue was re-dissolved in 5 ml of 3M trace metal analysis hydrochloric acid. 5 ml of deionised water was added to avoid corrosion of the analytical equipment, to decrease viscosity of the sample and to increase the amount of sample available for analysis. The sample was left to stand to allow deposits to dissolve. The sample was then filtered into a sample bottle and labelled.

4.2.3.2. Replication of samples

Samples were combined in the case of small fish to obtain 2 g of homogenised material, since preliminary analyses showed this to be an appropriate amount of tissue to enable measurements of most metals effectively. Two to five small individuals were amalgamated to provide one sample. Two to four replicate samples of 2 g per sample were prepared per fish for larger fish and the average concentration was calculated. This replication enabled the precision of the homogenisation and other aspects of preparation to be checked.

In most shrimp samples, unless the shrimp were very large, two whole shrimps were combined to obtain 2 g of sample. Two to four replicate samples of 2 g per sample were prepared per crab and the average concentration was calculated.

4.2.3.3 Procedures for analysis of zinc, copper, lead, cadmium, arsenic and chromium

Flame Atomic Absorption Spectrophotometry (FAAS) was used to determine Zn, Cu, Pb, Cd, As and Cr concentrations in fish and Crustacea. FAAS is based upon flame atomisation to measure the absorption and emission of radiation by excited atoms (Skoog *et al*, 1997). In flame atomisation, a solution of the metallic ions is nebulized (reduced to an aerosol or suspension of finely divided liquid particles in a gas) using a pneumatic nebulizer. The aerosol is carried into the burner flame by a flow of gaseous oxidant and fuel. The solvent evaporates in the primary combustion zone, which is located just above the tip of the burner. The resulting finely divided solid particles are carried to a region in the centre of the flame called the interzonal region. In the hottest part of the flame, gaseous atoms and elementary ions are formed from the solid particles. Emission and absorption spectra are generated in the resulting hot, gaseous medium.

A beam of absorbing wavelength is shone through the flame and into the entrance slit of the monochromator using a specific source hollow-cathode lamp per element. The monochromator is a component that isolates the required analytical wavelength of light emitted by the hollow cathode lamp. The lamp radiation passes through a modulator before it enters the flame and hence, interacts with the analyte. The photo-multiplier is the detector, providing adequate sensitivity over the required wavelength range. It responds to the intensity of the radiation from the monochromator and converts it to electrical energy which activates the display meter to read the absorption of the radiation of metals. FAAS is a precise and reliable method for obtaining quantitative data on metals in ionised form.

Attaining high precision requires careful calibration of the absorption response using standards to achieve accuracy. Four matrix matched calibration standards of known concentration per element were used (the standards were prepared by serial dilution of stock solutions to 1000 ppm). Calibration calculates average values from a line of best fit for each metal and the FAAS measures the concentration of the ion in each sample solution (parts per million). Each ion was analysed separately in accordance with the Varian cookbook

method. Zn, Cu, Pb, Cd and Cr were analysed using an air/acetylene flame and As was analysed using a acetylene nitrous oxide flame because a hotter flame is required for this element.

The concentration in solution samples was converted to the concentration of dry mass samples:

Concentration in sample ($\mu g\ g^{-1}$ dry mass) =

AAS reading x dilution factor per unit / dry mass of tissue (g)

The dilution factor was 10 for the solution of the sample. Zinc concentrations in the samples were above the range of the calibration curve so each sample was diluted with 1ml sample: 100ml of deionised water before analysis for Zn concentrations. The concentrations in the samples have been expressed as mg kg^{-1} dry mass in order to be comparable to the majority of published data and Environment Agency data.

Three FAAS machines were potentially available for the metal analysis in this study. Preliminary measurements and comparisons were made on two, a Perkin-Elmer 5000 and a Varian Spectra 2220FS. The machine subsequently used for all measurements given in the results was a Varian Spectra 220FS Atomic Absorption Spectrometer because it was regarded as the most precise on the basis of calibration and repeated measurements. Dogfish liver and dogfish muscle, containing certified metal concentrations, were used as reference materials to provide Quality Assurance. This reference material was treated and analysed under the same conditions as the fish samples for analysis. The results obtained were in good agreement with certified values.

4.2.4. Statistical methods

Statistical analysis was only conducted on the predominant species in seal diet, whiting, cod, flounder, plaice, sprat, herring, common shrimp and shore crab. Statistical tests of bi-monthly variation in metal concentrations were conducted for whiting, flounder, sprat and common shrimp since a larger number of samples were collected per season for these species (Appendix Ii) and these species represented the dominant prey consumed.

Comparison of winter (September-February) samples and summer (March-August) samples were conducted on cod, plaice, herring and shore crab (Appendix Iii).

4.2.4.1. Data Distribution and goodness of fit

Tests for skewness and kurtosis were performed on the metal concentrations data, using SPSS. Distributions of sampled metal concentrations were significantly right skewed and tended to be platykurtic. In addition, a test for equality of variances showed that the data was heteroscedastic. The data did not, therefore, meet the assumptions of parametric statistics. Logarithmic transformations were performed. This method is applicable when data is heteroscedastic and can usually convert a positively skewed distribution into a symmetrical distribution. Log transformation reduces the influence of extreme high values and often increases the power to detect significant correlations between metal concentrations and body size. All the elements are put on a common scale that facilitates comparisons between metal concentrations and proportional change in metal concentrations with body size.

The Kolmogorov-Smirnov statistic was used to test the goodness of fit for normal distribution and log-normal distribution for variables of size measurements and metal concentrations for all species. Not all of the data was normally distributed even after the data was logged. Non-parametric tests were used to analyse the strongly skewed non-transformed data through out Chapter 4.

4.2.4.2. Coefficients of variance (CV)

The essential metals, Zn and Cu are expected to vary less than non-essential metals due to regulation at metabolically required levels (Bryan, 1968; Thompson, 1990; Kress *et al*, 1999). Coefficient of variation of log transformed metal concentrations was used to compare the extent of variability in levels of metals for each species. The calculation was:

Coefficient of variance (CV) = standard deviation / mean.

4.2.4.3. Comparison of metal concentrations

Metal concentrations were compared between each of species, bi-monthly periods, winter and summer and years. Box plots were used to summarize the comparisons between species and between bi-monthly periods. Boxplots were the appropriate graphical method for displaying strongly skewed data because they do not require normally distributed data (Dytham, 2003). Each data sample is represented by a box whose top and bottom represent the lower and upper quartiles of the data. The box is divided by the median value. The whisker represents values between the upper quartile and the largest values within interquartile ranges of the top and values between the lower quartile and the smallest values within interquartile ranges of the bottom. Values outside of this range are outliers and extreme values and are identified with symbols.

The non-parametric test, Kruskal Wallis H test, was used to compare the difference between metal concentrations for each of species, bi-monthly periods and years. Where there was a significant difference in metal concentrations between any of these variables, Mann-Whitney U test was applied to determine which pairs of variables were different.

Principal Component Analysis (PCA) was applied to investigate the relationships for each metal between species using the Community Analysis Package software. Although PCA assumes that data is normally distributed, whereas some data in this study was not lognormal, it is possible to overlook this if the purpose of the test is to generate further hypotheses (Dytham, 2003). PCA transforms the data into orthogonal components which are linear combinations of the original variables.

4.2.4.4. Correlation between metal concentrations

Spearman rank correlation was used to analyse, the interaction between pairs of metal concentrations within each species, the correlation between body size and metal concentrations, the comparable correlations between body size and metal concentrations for each bi-monthly period and the comparable correlations between body size and metal

concentrations for winter and summer. The three parameters of body size used were length, dry mass and wet mass.

The relationship between body size and metal concentrations may be confounded by seasonality. Variations in metal concentrations with body size can be adjusted for in order to test different cohorts of fish for similarity in metal concentrations. This is particularly important when detecting changes over time with potentially different pollution exposures. The influence of a continuous measurable characteristic, such as body length or mass, can be removed by analysis of covariance on lognormal metal concentrations. In this context ANCOVA requires significant correlations between body size and metal concentrations. Cases of significant correlations were not consistent throughout the data so ANCOVA was not an appropriate technique. In addition, ANCOVA is a parametric statistic and since some of the data did not exhibit normal or lognormal distributions this method was not applicable. Spearman correlation coefficient was therefore used to test the correlation between body size and metal concentrations of common shrimp, whiting, flounder, sprat and herring for each bi-monthly season and the correlation between body size and metal concentrations for these species, plus the shore crab between each of winter and summer.

4.2.5. Materials and methods for analysis of mercury concentrations

The fish species collected for Hg analysis were whiting and flounder. These species were analysed because they were the two dominant prey species in seal diet and represent pelagic (whiting) and benthic (flounder) lifestyles. Samples were collected in the summer (July-August) and winter (November-December) for both species (Appendix Iiii).

4.2.5.1. Digestion techniques

Samples for Hg analysis were digested using a CEM Mars SX microwave. The method used was EPA 3052. Closed vessels were used for analysis of Hg, as this helps to avoid loss, since Hg is a particularly volatile element. Approximately 0.2 g of homogenised sample was placed in acid washed Teflon microwave digestion vessels and the mass noted. The sample was oxidised to break down organic matter by wet digestion using 5 ml double

distilled water and 2ml trace metal grade peroxide (100 vol.), then 10 ml trace metal grade nitric acid, 2 ml trace metal grade hydrochloric acid, 2 ml trace metal grade hydrofluoric acid (48%) and 1 ml of 1000 ppm gold standard. Gold was added to enhance the signal which is standard practice for all ICP Hg analysis. The substances were added separately and at each stage it was necessary to wait for the reaction between the sample and the substance to calm before adding the next substance. The solution was filtered into a plastic conical flask and made up to 100 ml using double distilled water. It was then transferred to a plastic sample bottle and labelled.

4.2.5.2. Replication of samples

Two replicate samples of 0.2 g per sample were prepared for six flounder and 19 whiting and three replicate samples of 0.2 g per sample were prepared for two whiting. The average Hg concentration was calculated. The precision of homogenisation was checked using this replication and dogfish liver and dogfish muscle, containing certified Hg concentrations, was used as reference material.

4.2.5.3. Procedures for analysis of mercury

The digested samples were diluted by a factor of 1000 in steps and analysed on a Perkin-Elmer Elan 6100 DRC. The limits of detection were 1 mg kg^{-1} dry mass. Five matrix matched calibration standards of 50, 100, 250, 500 and 1000 ppb were used (the standards were prepared by serial dilution of stock solutions to 1000 ppm). Analytical accuracy was assessed using dogfish liver and dogfish muscle, containing certified Hg concentrations, as reference materials. This reference material was treated and analysed under the same conditions as the fish samples for analysis. The results obtained were in good agreement with certified values.

4.2.5.4. Statistical analysis

There were too few samples where Hg was detected to allow for statistical comparisons.

4.3 RESULTS OF ZINC, COPPER, LEAD, CADMIUM, ARSENIC AND CHROMIUM ANALYSIS IN CRUSTACEA AND FISH FROM THE TEES ESTUARY, 1999-2002

The results of Zn, Cu, Pb, Cd, As and Cr analysis in Crustacea and fish species are presented first, followed by Hg analysis (Section 4.4). Hg analysis was conducted on whiting and flounder only.

4.3.1. Metal concentrations in Crustacea and fish species from the Tees Estuary

Zinc, Cu, Pb, Cd, As and Cr were analysed in Crustacea and fish species. The results are presented as medians and inter-quartile range (Table 4.2). Median metal concentrations were mostly higher in Crustacea than fish, with the exceptions of relatively low Zn concentrations in crabs, high Zn concentrations in pleuronectids and relatively high median concentrations of As and Cr in plaice.

Median Cu, Pb, Cd and Cr concentrations were lowest in lesser sandeels, whereas Zn concentrations were relatively high in lesser sandeel. Metal concentrations were relatively low in gadids, with the exception of relatively high Cu concentrations in saithe and relatively high Cr concentrations in cod. Four groups of As concentrations in fish were evident: plaice (19.2 mg kg^{-1}), sprat and herring (9.6 – 9.9 mg kg^{-1}), cod, flounder and whiting (7.8 – 8.3 mg kg^{-1}) and lesser weever, lesser sandeel and saithe (4.9–5.2 mg kg^{-1}).

Table 4.2. Median metal concentrations in Crustacea and fish species (with twenty-five percentiles and n = number in sample) (mg kg^{-1} dry mass) (see glossary for abbreviations of species names)

	Zn	Cu	Pb	Cd	As	Cr
Sh. crab	87.2 (n=128)	22.7 (n=128)	11.3 (n=129)	0.9 (n=129)	12.3 (n=109)	3.1 (n=128)
	76.2-107.6	15.5-30.3	8.6-14.1	0.7-1.1	8.5-18.8	2.5-3.8
Sw.crab	86.8 (n=36)	24.1 (n=36)	9.6 (n=36)	0.9 (n=36)	13.5 (n=33)	2.8 (n=36)
	69.0-112.0	18.3-40.7	7.5-13.7	0.6-1.2	10.3-23.9	1.6-3.9
C. Shr	135.7 (n=157)	41.7 (n=157)	8.0 (n=178)	0.7 (n=177)	21.4 (n=157)	1.6 (n=161)
	111.0-169.5	30.8-51.0	6.1-10.4	0.5-0.9	13.7-28.0	1.1-2.5
Prawn	113.2 (n=7)	80.5 (n=7)	5.0 (n=7)	0.5 (n=7)	40.3 (n=7)	1.5 (n=7)
	77.4-132.7	36.7-135.5	2.2-7.2	0.4-0.7	28.6-52.2	1.1-1.7
All fish	**86.1 (n=1163)**	**2.2 (n=1173)**	**2.9 (n=1155)**	**0.3 (n=1156)**	**8.1 (n=1051)**	**0.9 (n=725)**
species	**58.9 - 120.2**	**1.6 - 3.1**	**2.0 - 4.3**	**0.2 - 0.5**	**4.7 - 14.5**	**0.7 - 1.4**
Wh	61.8 (n=262)	1.9 (n=262)	2.6 (n=257)	0.3 (n=258)	7.8 (n=231)	0.9 (n=191)
	48.6 - 81.9	1.5 - 2.5	1.7 -3.6	0.1 -0.4	4.7 -14.3	0.7-1.4
Cod	61.1 (n=39)	1.9 (n=39)	3.2 (n=39)	0.2 (n=38)	8.3 (n=34)	1.2 (n=35)
	49.7 -78.8	1.5 -2.5	2.7 - 4.5	0.2 - 0.3	4.7 -10.4	0.9 -1.7
Sai	66.3 (n=35)	2.6 (n=35)	2.9 (n=34)	0.3 (n=35)	4.9 (n=30)	0.8 (n=21)
	52.8 -85.8	2.0 -3.6	2.0 - 4.0	0.2 -0.4	3.0 -11.1	0.5 -1.4
Fl	115.5 (n=245)	2.4 (n=246)	3.7 (n=247)	0.4 (n=248)	7.9 (n=228)	1.1 (n=161)
	91.6 -142.6	1.9 -3.4	2.6 -5.2	0.3 -0.5	4.8 -14.0	0.8 -1.7
Pl	97.2 (n=35)	2.2 (n=36)	4.3 (n=36)	0.3 (n=35)	19.2 (n=32)	1.6 (n=15)
	67.1 -121.3	1.8 -2.7	2.7 -6.0	0.2 -0.5	12.7 -31.5	1.0 -2.5
Sp	93.2 (n=244)	2.6 (n=246)	2.9 (n=241)	0.3 (n=239)	9.6 (n=192)	0.9 (n=137)
	57.8 -133.9	1.5 -3.6	1.8 -4.4	0.1 -0.6	5.5 -15.4	0.6 -1.4
Herr	91.8 (n=154)	2.6 (n=156)	2.9 (n=150)	0.4 (n=153)	9.9 (n=131)	1.0 (n=85)
	60.1 -122.3	1.7 -3.3	2.0 - 4.4	0.2 -0.6	5.0 -15.8	0.7 -1.3
Weev	77.8 (n=66)	2.4 (n=68)	3.1 (n=68)	0.4 (n=67)	5.0 (n=60)	1.0 (n=44)
	58.8 -107.5	1.8 -2.9	2.3 -3.9	0.3 -0.4	3.3 -7.7	0.6 -1.4
SE	92.4 (n=41)	1.6 (n=41)	1.7 (n=40)	0.2 (n=41)	5.2 (n=35)	0.5 (n=32)
	78.9 -139.4	1.1 -2.2	0.9 -3.3	0.2 -0.3	3.2 -7.7	0.2 -0.8

4.3.1.1. Comparison of metal concentrations between crustacean species from the Tees Estuary

There was a very highly significant difference in metal concentrations between common shrimp and shore crab ($p < 0.001$) (Mann Whitney U test). Zn, Cu and As concentrations were highest in common shrimp, whereas Pb, Cd and Cr concentrations were highest in the shore crab. This same pattern is shown with higher Zn, Cu and As in prawns than in swimming crabs but sample sizes were too small to be statistically significant.

4.3.1.2. Comparison of metal concentrations between fish species from the Tees Estuary

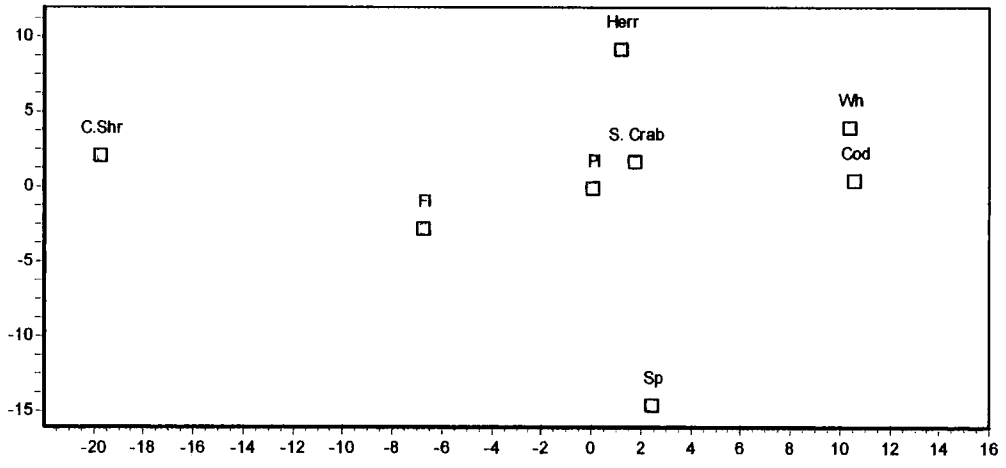
Median metal concentrations were compared between fish species. There was a statistically significant difference in metal concentrations between whiting cod, flounder, plaice, sprat and herring for all six metals using Kruskal Wallis ($p<0.001$). Differences in pairs of species were compared for each metal (Mann Whitney U test) (Appendix J). The sample size for each species is large and may lead to Type I errors. That is the null hypothesis is rejected when it was actually true and there was no difference in metal concentrations between species. To control for Type I errors only a significance level of $p < 0.001$ was accepted as statistically significant and the difference in metal concentrations between species are shown graphically (Appendix K).

Zinc, Cu and Cd concentrations were higher in the pleuronectid and clupeid species and lowest in the gadid species. Pb concentrations were highest in the pleuronectid species and lowest in whiting but there was no significant difference between Pb concentrations in the cod and pleuronectid and clupeid species. Cr concentrations were highest in the pleuronectid species and cod and lowest in whiting and clupeid species. The only significant differences in As concentrations were higher concentrations in plaice than flounder and cod and higher concentrations in sprat than flounder.

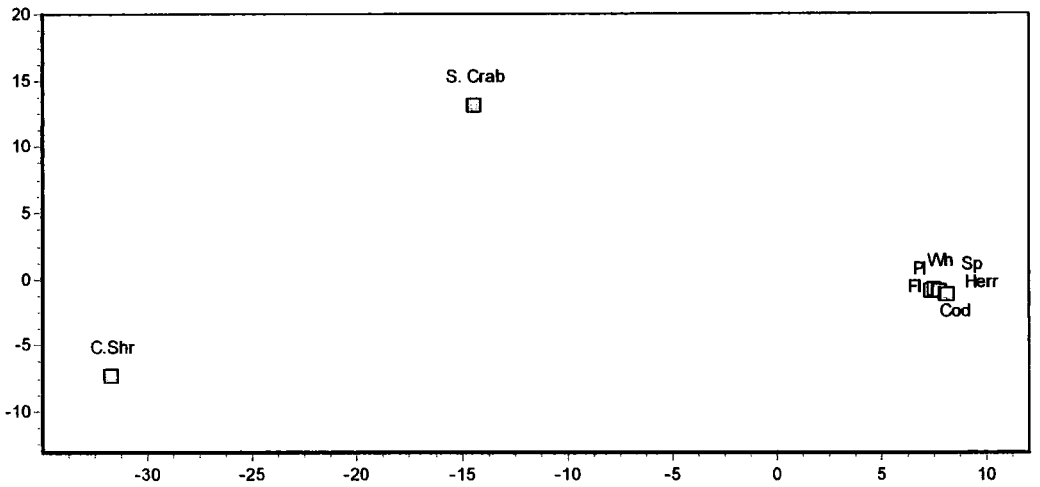
Chromium concentrations were significantly higher in plaice than in flounder. Pb and Cr concentrations were significantly higher in cod than whiting. There were no significant differences in metal concentrations between sprat and herring.

Principal component analysis (PCA) was conducted for each metal to observe the relationship in metal concentrations between the species (Figure 4.1 a-f). There was a correlation for Zn concentrations on axis 1 between whiting and cod and a correlation between all other species except common shrimp and on axis 2 there was a correlation between all fish except herring and sprat with were at opposite ends of the axis. There was a correlation for copper and lead concentrations on axis 1 and axis 2 between Crustacea and a correlation between all fish species. There was also a correlation for cadmium concentrations on axis 1 between Crustacea and a correlation between all fish species but there was no correlation on axis 2. There was a correlation for arsenic concentrations on axis 1 between plaice and common shrimp and a correlation between all other species. There was a correlation for chromium concentrations on axis 1 between all other species, except shore crab and there was a correlation for chromium concentrations on axis 2 between species except plaice and common shrimp which were at opposite ends of the axis.

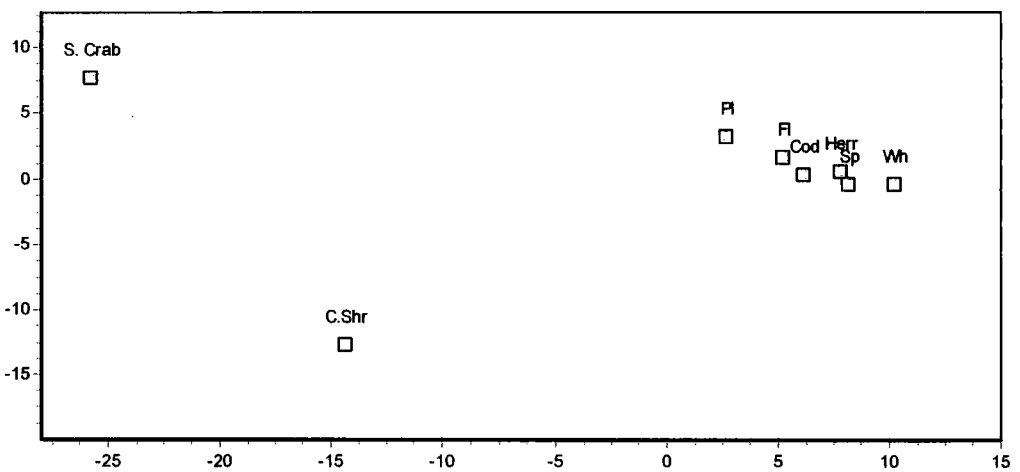
a)



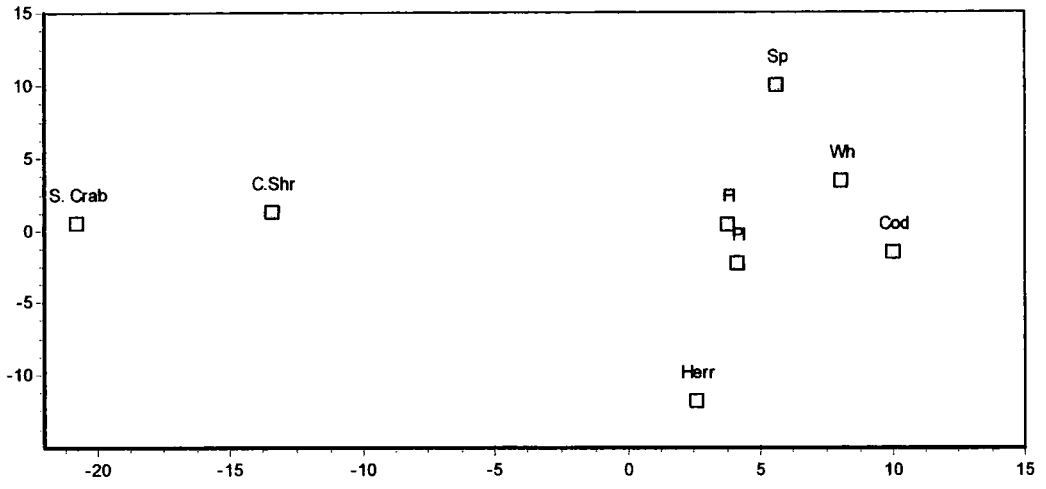
b)



c)



d)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S. Crab
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Cod
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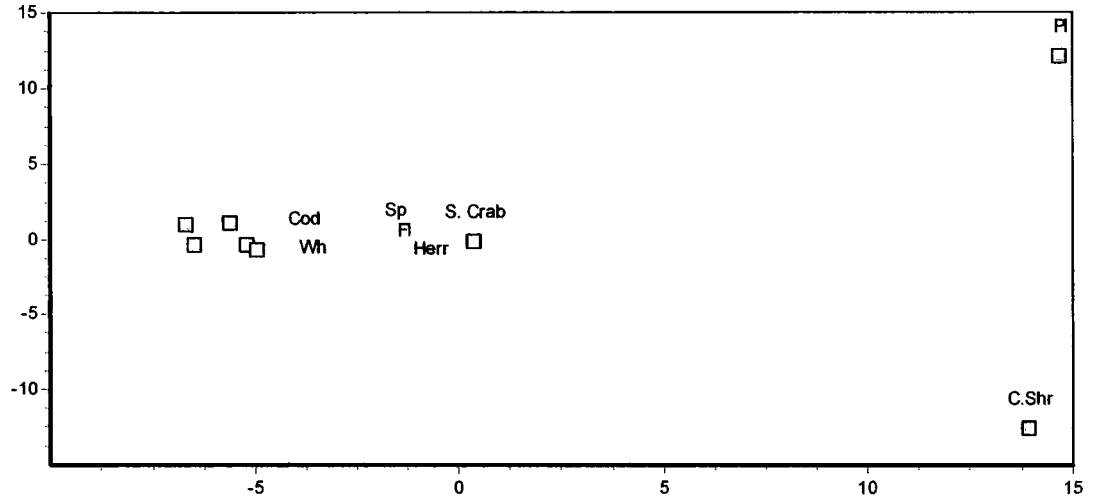
e)


15
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f)

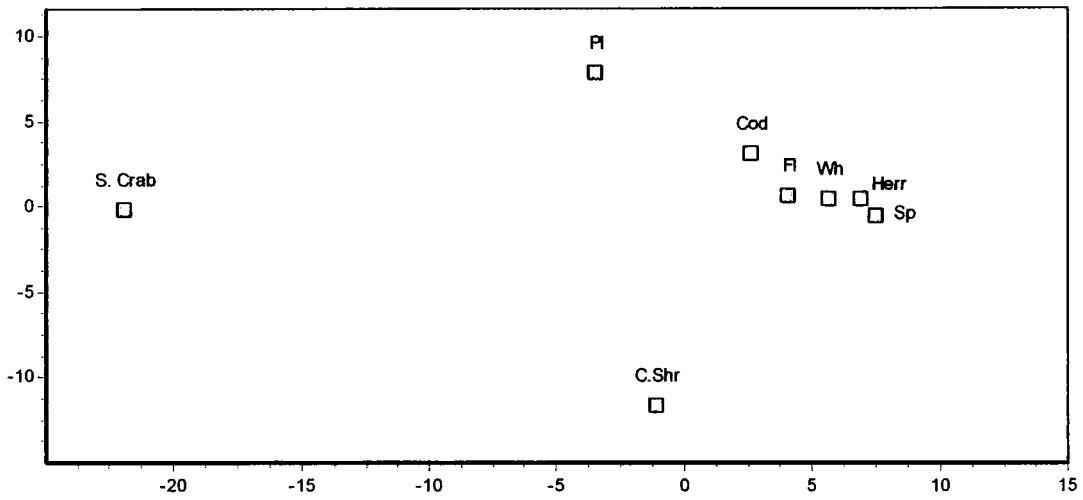


Figure 4.1. PCA plot of the correlation between metal concentrations in each species a) zinc b) copper c) lead d) cadmium e) arsenic f) chromium

4.3.2. Variation of metal concentrations between Crustacea and fish species from the Tees Estuary

Coefficients of variance (CV) were used to compare variation between log transformed metal concentrations at the same magnitude (Figure 4.2).

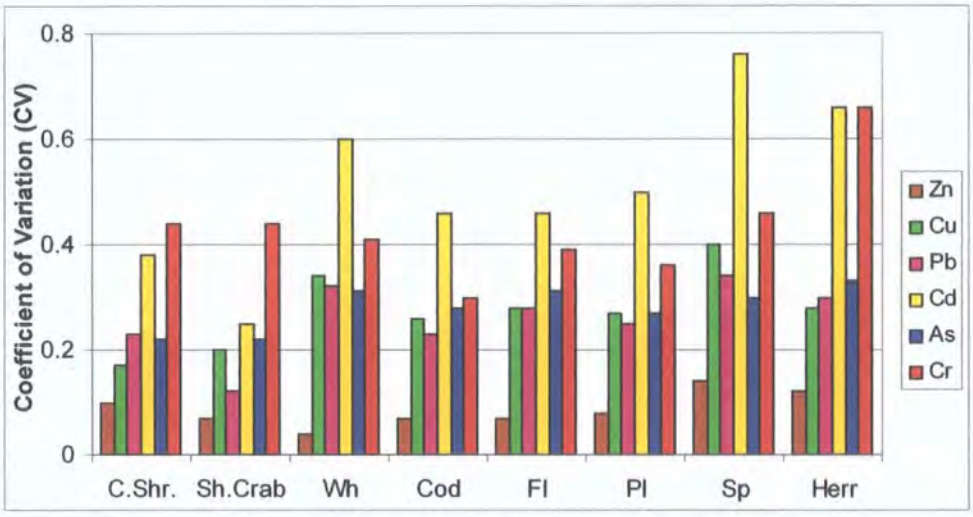


Figure 4.2. The coefficients of variation of Zn, Cu, Pb, Cd, As and Cr in species of Crustacea and fish (see glossary for abbreviations)

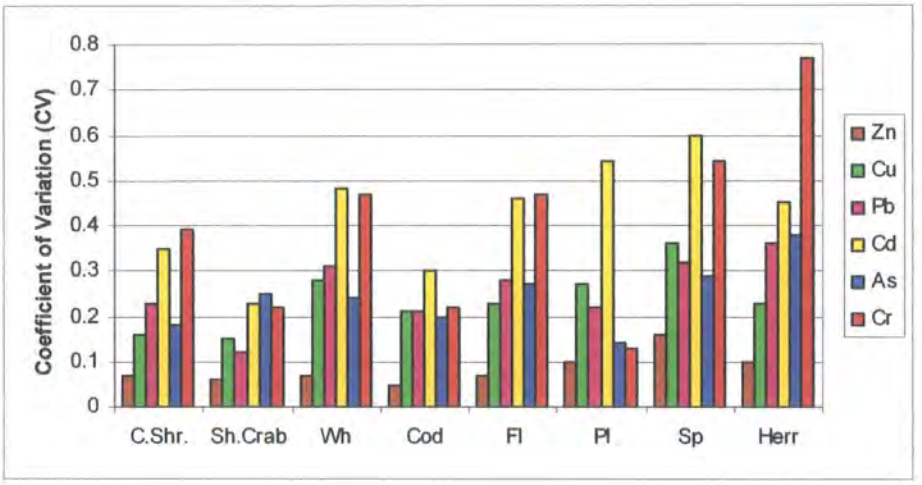
The highest to lowest ranks of coefficients of variation within each species were:

Common shrimp = Cr > Cd > Pb > As > Cu > Zn
Shore crab = Cd > Cr > As > Cu > Pb > Zn
Whiting = Cd > Cr > Cu > Pb > As > Zn
Cod = Cd > Cr > As > Cu > Pb > Zn
Flounder = Cd > Cr > As > Cu = Pb > Zn
Plaice = Cd > Cr > Cu = As > Pb > Zn
Sprat = Cd > Cr > Cu > Pb > As > Zn
Herring = Cr = Cd > As > Pb > Cu > Zn

Variation was lowest in the essential metal Zn and highest in either Cd or Cr. The variation in the essential metal Cu was relatively intermediate in most species, although low in shrimp and herring.

Seasonal differences in coefficients of variance (CV) were compared between winter and summer samples to determine whether variation between log transformed metals differed seasonally (Fig 4.3.a and b).

a)



b)

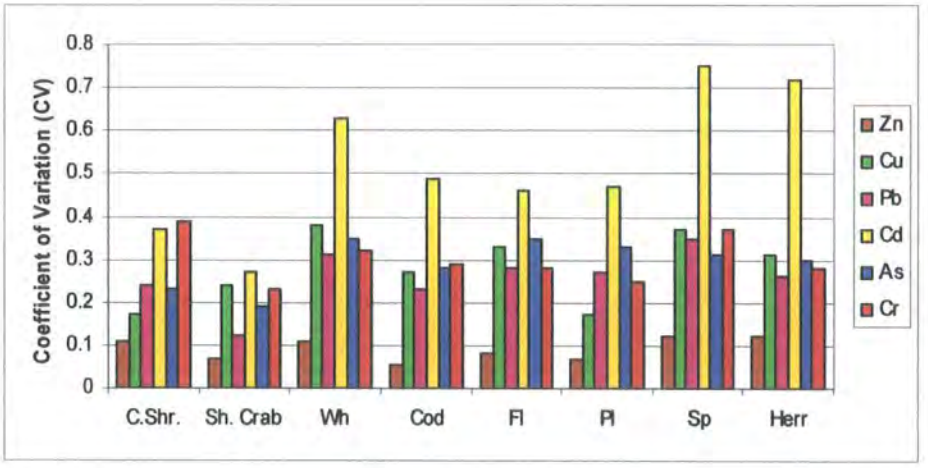


Figure 4.3. Seasonal coefficients of variation of Zn, Cu, Pb, Cd, As and Cr in species of Crustacea and fish a) winter b) summer (see glossary for abbreviations)

There were no statistically significant difference in metal variations between winter and summer for any species (Mann-Whitney U-test), despite the appearance of some changes in the amount of variation between winter and summer (Figure 4.3).

4.3.3. Interaction between metal concentrations in Crustacea and fish from the Tees Estuary

In the case of one metal having a high concentration for any given individual crustacean or fish then all metals might be expected to have high concentrations in that individual, given that they may have similar routes of uptake and sources of exposure. Conversely, in the case of one metal having a low concentration in an individual Crustacea or fish then all metals might be expected to have low concentrations in that individual. Correlation analyses were conducted between the six metal concentrations for each species (Table 4.3). Where strong correlations of greater than 0.6 are observed they are between Cd and Pb or Cu, or in one case As.

Table 4.3. Spearman's correlation between metal concentrations i) common shrimp ii) shore crab iii) whiting iv) cod v) flounder vi) plaice vii) sprat viii) herring

i) common shrimp

Zn					
0.342***	**Cu**				
n=157					
0.221**	0.429***	**Pb**			
n=156	n=178				
0.419***	0.621***	0.620***	**Cd**		
n=156	n=177	n=176			
0.320***	0.590***	0.365***	0.624***	**As**	
n=154	n=157	n=156	n=155		
0.414***	0.294***	NS	0.396***	0.315***	**Cr**
n=154	n=161		n=159	n=154	

ii) shore crab

Zn					
0.292***	**Cu**				
n=127					
NS	0.250**	**Pb**			
	n=128				
NS	0.303***	0.718***	**Cd**		
	n=128	n=129			
NS	NS	NS	NS	**As**	
NS	0.435***	0.731***	0.665***	0.228 *	**Cr**
	n=127	n=128	n=159	n=108	

iii) whiting

Zn					
0.251 *** n=262	**Cu**				
0.179 ** n=257	0.483 *** n=257	**Pb**			
0.280 ***n=258	0.672 *** n=258	0.491 *** n=253	**Cd**		
NS	0.460 *** n=231	0.362 *** n=226	0.428 *** n=228	**As**	
0.263 *** n=191	NS	0.197 ** n=190	NS	0.160 * n=166	**Cr**

iv) cod

Zn					
0.404 * n=39	**Cu**				
NS	NS	**Pb**			
0.366 * n=38	0.347 * n=38	0.619 *** n=38	**Cd**		
NS	NS	NS	0.488 ** n=33	**As**	
NS	NS	0.472 ** n=35	0.592 *** n=35	NS	**Cr**

v) flounder

Zn					
0.339 *** n=243	**Cu**				
0.258 *** n=244	0.531 *** n=245	**Pb**			
0.230 *** n=245	0.507*** n=246	0.486 *** n=247	**Cd**		
NS	0.260 *** n=226	0.231 *** n=227	NS	**As**	
0.210 ** n=159	NS	NS	NS	NS	**Cr**

vi) plaice

Zn					
NS	**Cu**				
NS	NS	**Pb**			
NS	0.333 * n=35	0.395 * n=35	**Cd**		
NS	0.471 ** n=32	0.456 ** n=32	NS	**As**	
NS	NS	NS	NS	NS	**Cr**

vii) sprat

Zn					
0.436 ***	**Cu**				
n=243					
0.301 ***	0.553 ***	**Pb**			
n=238	n=240				
0.315 ***	0.682***	0.691 ***	**Cd**		
n=237	n=238	n=233			
NS	0.420 ***	0.400 ***	0.373 ***	**As**	
	n=191	n=188	n=188		
0.511 **	0.276 ***	0.216 *	0.317 ***	NS	**Cr**
n=136	n=136	n=132	n=134		

viii) herring

Zn					
0.196 *	**Cu**				
n=154					
NS	0.570 ***	**Pb**			
	n=150				
NS	0.624***	0.710 ***	**Cd**		
	n=153	n=148			
NS	0.453 ***	0.289 ***	0.495 ***	**As**	
	n=131	n=188	n=130		
0.496 **	0.341 ***	0.464 ***	0.356 ***	NS	**Cr**
n=84	n=85	n=81	n=83		

4.3.4. Seasonal metal concentrations in Crustacea and fish from the Tees Estuary

Statistical difference between bi-monthly metal concentrations common shrimp, whiting, flounder and sprat are shown (Table 4.4). This is shown graphically in Appendix Li - Liv.

Table 4.4. Comparison of metal concentrations between bi-monthly periods in common shrimp, whiting, flounder and sprat (Kruskal Wallis H test)

	Zn	Cu	Pb	Cd	As	Cr
Common shrimp	$p<0.001$	$p<0.001$	$p<0.05$	$p<0.001$	$p<0.001$	$p<0.001$
Whiting	NS	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.05$
Flounder	$p<0.001$	$p<0.01$	$p<0.001$	$p<0.01$	NS	$p<0.05$
Sprat	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$

Metal concentrations in common shrimp, whiting and flounder tended to be higher in summer months than winter months for all metals. The exceptions were Zn in the whiting tissues and As in flounder tissues, which did not show any significant difference. Metal concentrations tended to be higher in summer months than winter months for the sprat, although Cu, Pb, Cd and As concentrations were relatively high in January-February compared to other winter months and Cr concentrations were relatively high in September-October compared to the other winter months.

4.3.5. Comparison between winter (September-February) and summer (March-August) metal concentrations in Crustacea and fish species from the Tees Estuary

Winter and summer metal concentrations in the biota of the Tees Estuary were compared (Table 4.5).

Table 4.5. Significant differences between winter and summer metal concentrations of species from the Tees Estuary (Mann-Whitney U Test)

Species	Metal	p-value	Season with highest value
Shore crab	Cd	< 0.05	Summer
Whiting	Cu	< 0.001	Winter
	As	< 0.001	Winter
	Cd	< 0.001	Summer
Flounder	Cu	< 0.001	Summer
	Cd	< 0.001	Summer
	Pb	< 0.05	Summer
Plaice	Cr	< 0.001	Summer
	Cu	< 0.05	Summer
Sprat	Zn	< 0.001	Summer
	Cu	< 0.001	Summer
	Pb	< 0.001	Summer
	Cd	< 0.001	Summer
	As	< 0.05	Winter
Herring	Zn	< 0.001	Winter
	As	< 0.01	Winter

Where there was a significant difference this tended to be for higher levels in the summer, as tended to be the case for the bi-monthly samples.

4.3.6. Correlation between body size and heavy metal concentrations in Crustacea and fish from the Tees Estuary

Correlations between body size and metal concentrations are shown (Table 4.7). Three parameters of body size were compared: length, dry mass and wet mass. The significant negative correlations between body size and metal concentrations tend to indicate higher metal concentrations in small biota but the correlations are generally not strong, as there was a large degree of individual variability in body concentrations, especially in small to medium-sized biota. The large sample sizes may have lead to Type I errors. Significant correlations were regarded as those greater than 0.7 and very highly significant ($p<0.001$). There were very highly significant negative correlations between the body size of cod and plaice and Cr concentrations (Table 4.6).

Table 4.6. Correlations between metal concentrations and a) length, b) dry body mass and c) wet body mass in crustaceans and fish (Spearman's Correlation Coefficient)

a) length

	Zn	Cu	Pb	Cd	As	Cr
Common shrimp	-0.28 ***	NS	-0.23 **	-0.24 ***	NS	-0.29 ***
Shore crab	NS	-0.20 *	-0.29 ***	-0.18 *	NS	-0.31 ***
Whiting	NS	-0.27 ***	-0.23 ***	-0.22 ***	NS	-0.36 ***
Cod	NS	NS	-0.35 *	-0.44 **	NS	-0.73 ***
Flounder	-0.19 ***	NS	NS	NS	NS	NS
Plaice	-0.54 ***	NS	NS	NS	NS	-0.74 **
Sprat	-0.36 ***	-0.36 ***	-0.33 ***	-0.31 ***	-0.19 **	-0.41 ***
Herring	-0.32 ***	-0.16 *	-0.27 ***	-0.24 **	-0.41 ***	-0.40 ***

b) dry body mass

	Zn	Cu	Pb	Cd	As	Cr
Common shrimp	NS	NS	NS	NS	NS	-0.24 **
Shore crab	NS	NS	NS	NS	NS	NS
Whiting	NS	-0.32 ***	-0.23 ***	-0.27 ***	-0.16 *	-0.35 ***
Cod	NS	NS	-0.39 *	-0.42 **	NS	-0.76 ***
Flounder	-0.23 ***	-0.14 *	NS	NS	NS	NS
Plaice	-0.56 ***	NS	NS	NS	NS	-0.82 ***
Sprat	-0.54 ***	-0.49 ***	-0.39 ***	-0.38 ***	-0.23 **	-0.44 ***
Herring	-0.40 ***	-0.26 ***	-0.30 ***	-0.28 ***	-0.44 ***	-0.47 ***

c) wet body mass

	Zn	Cu	Pb	Cd	As	Cr
Common shrimp	-0.25 **	NS	-0.22 **	-0.24 ***	NS	-0.27 ***
Shore crab	NS	-0.20 *	-0.32 ***	-0.27 **	NS	-0.37 ***
Whiting	NS	-0.25 ***	-0.21 ***	-0.20 ***	NS	-0.36 ***
Cod	NS	NS	-0.36 *	-0.44 **	NS	-0.72 ***
Flounder	-0.22 ***	NS	NS	NS	NS	NS
Plaice	-0.53 ***	NS	NS	NS	NS	-0.73 **
Sprat	-0.38 ***	-0.36 ***	-0.31 ***	-0.32 ***	-0.15 *	-0.48 ***
Herring	-0.35 ***	-0.22 **	-0.29 ***	-0.27 ***	-0.43 ***	-0.46 ***

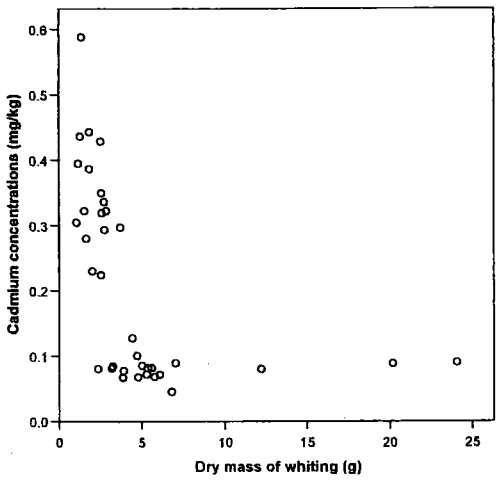
4.3.7. Seasonal affect of body size on metal concentrations in Crustacea and fish from the Tees Estuary

The correlation between body size and metal concentrations is generally negative and metal concentrations tend to be higher during the summer. This may be explained by a higher incidence of small individuals (mainly juvenile) of each species in the summer. The exceptions when concentrations were higher in the winter were As concentrations in whiting, sprat and herring, Cu concentrations in whiting and zinc concentrations in herring. Variations in body size with seasonality were shown graphically (Appendix Mi-v and Ni-v). The median dry mass of common shrimp and sprat was slightly lower during the summer. There was no difference in seasonal dry mass for shore crab and plaice. Whiting and flounder both had similar median dry mass in summer and winter but larger individuals above the median in winter. The dry mass of herring was higher during the summer.

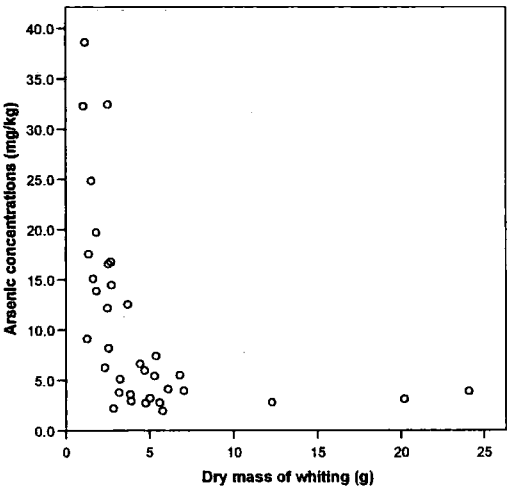
The correlation between body size and metal concentrations of common shrimp, whiting, flounder, sprat and herring for each bi-monthly season is shown (Appendix Oi - Ov) and examples of the strongest negative correlations are shown (Figure 4.4). If the negative correlation between body size and metal concentrations was due to seasonal body size differences then negative correlations would not be expected between bi-monthly body size and metal concentrations. There were some negative correlations between body size and metal concentrations within bi-monthly periods for all species but no clear pattern for any particular metal.

Correlations between body size and metal concentrations of common shrimp, shore crab, whiting, flounder, sprat, herring, lesser weever and lesser sandeel between each of winter and summer were assessed and are presented in Appendix Pi-Pvi. There were some negative correlations between body size and metal concentrations in winter and summer samples but no clear pattern for any particular metal or species.

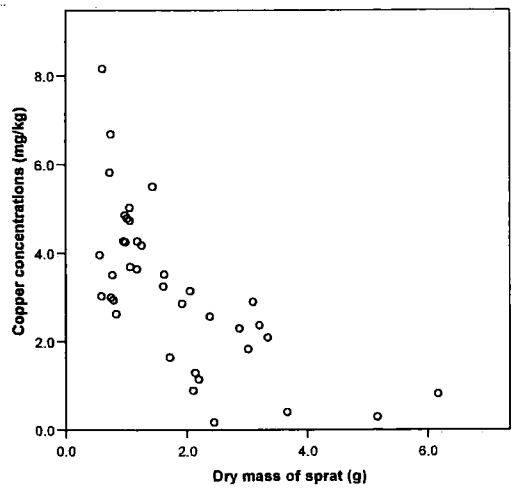
a)


Cadmium concentrations (mg/kg)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
5
10
15
20
25
Dry mass of whiting (g)

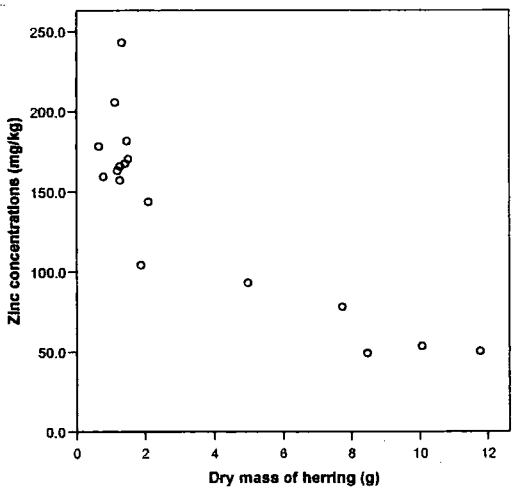
b)


Arsenic concentrations (mg/kg)
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
0
5
10
15
20
25
Dry mass of whiting (g)

c)


Copper concentrations (mg/kg)
0.0
2.0
4.0
6.0
8.0
0.0
2.0
4.0
6.0
Dry mass of sprat (g)

d)


Zinc concentrations (mg/kg)
0.0
50.0
100.0
150.0
200.0
250.0
0
2
4
6
8
10
12
Dry mass of herring (g)

e) f)

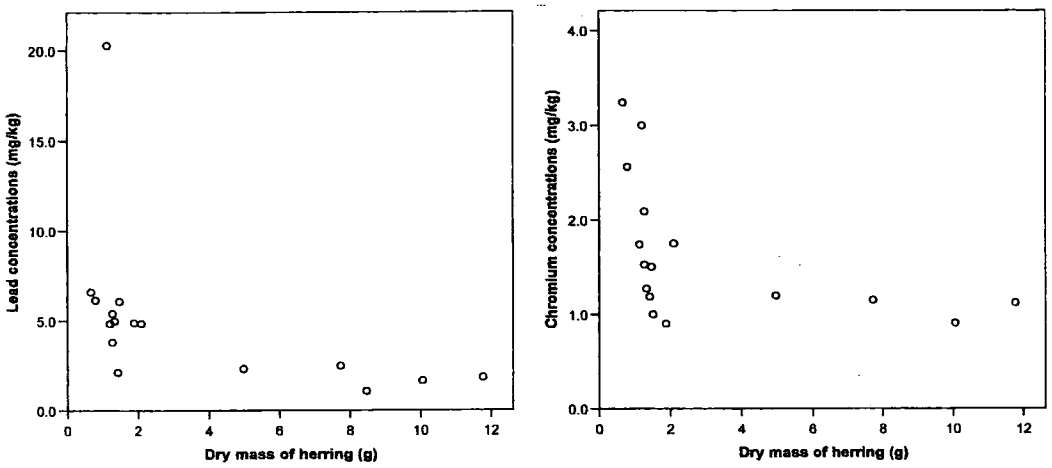


Figure 4.4. Examples of the strongest negative correlations between a) dry mass and cadmium concentrations for whiting in January-February, b) dry mass and arsenic concentrations for whiting in January-February, c) dry mass and copper concentrations for sprat in March-April, d) dry mass and zinc concentrations for herring in July-August, e) dry mass and lead concentrations for herring in July-August, f) dry mass and chromium concentrations for herring in July-August

4.4. RESULTS OF MERCURY ANALYSIS IN WHITING AND FLOUNDER TISSUES FROM THE TEES ESTUARY, 1999-2002

Mercury concentrations were below the limits of detection (1 mg kg^{-1} dry mass) in all flounder and 92 out of 101 whiting. Hg concentrations in the nine remaining whiting samples were detected at 2 mg kg^{-1} dry mass (Table 4.7). The percentage of Hg detected was lower in whiting samples collected in the summer than in the winter.

Table 4.7. Seasonal number and percentage of whiting samples with Hg not detected (ND) and Hg detected (2 mg kg^{-1} dry mass)

Season	No. of samples with ND	No. of samples with 2 mg kg^{-1} dry mass	Total
Winter	66 (89%)	8 (11%)	**74**
Summer	26 (96%)	1 (4%)	**27**
Total	**92**	**9**	**101**

The body sizes of the nine whiting samples with 2 mg kg^{-1} dry mass of Hg detected were within the range of body sizes of whiting where no Hg was detected but tended to have slightly higher median dry mass and wet mass (Table 4.8).

Table 4.8. Comparison of median body size of whiting with no Hg detected and Hg detected (2 mg kg^{-1} dry mass)

	0 mg kg^{-1} dry mass	2 ppm mg kg^{-1} dry mass
Dry mass	4.9	5.6
Wet mass	22.5	23.7
Length	145.0	145.4
No. of Samples	92	9

4.5. DISCUSSION OF METAL CONCENTRATIONS IN FISH AND CRUSTACEA FROM THE TEES ESTUARY

Differences in Zn, Cu, Pb, Cd, As and Cr concentrations were statistically analysed between two species of Crustacea and six species of fish. Whiting, cod, flounder, plaice, sprat, herring, common shrimp and shore crab were chosen as the species for the main analysis because they are present in the Tees Estuary in relatively large numbers and they are important prey species for seals and cormorants. In addition, they represent a range of lifestyles that influence habitat, diet, bioavailability and exposure to metal concentrations. Only a limited number of samples could be analysed for Hg so the two dominant fish species in seal and cormorant diet, whiting and flounder, were chosen. These two species can be used to compare the different lifestyles of the flounder which is an estuarine resident living in or on the substratum and the whiting which is marine juvenile migrant species living in the water layer just above the bed (Elliott and Dewailly, 1995).

4.5.1. Metal concentrations between species

Metal concentrations in this study were compared with metal concentrations measured in common shrimp, flounder and sprat from the Tees Estuary by the EA, 1998-2002 (Table 4.9 and 4.10). The EA has measured metal concentrations in common shrimp since 1986 and in flounder and sprat since 1994 but earlier concentrations were not included because they were expressed as wet mass. Zinc, Cu and Cr concentrations were expected to be lower in the present study than the EA study since concentrations declined slightly in the surrounding water and the sediment of the Tees Estuary between 1990 to 1997 and 1992 to 2002, respectively (Table 1.1 and Figure 1.1 a-b). Lead and Cd concentrations remained constant in the surrounding water of the Tees Estuary between 1997 and 2002 (Table 1.1) and Pb concentrations in the sediment declined slightly (Figure 1.1 c), whilst Cd concentrations remained relatively constant (Figure 1.1 d). Arsenic concentrations in the sediment increased by 1995 but then remained constant (Chapter 1, Figure 1.1 e) so concentrations in the biota are expected to be either constant or higher in the present study than the EA study Cd concentrations are therefore expected to remain constant between the

two studies, whilst Pb concentrations may be expected to stay constant or to be lower in the present study than the EA study depending on whether Pb in the water body or the sediment influences metal concentrations in common shrimp, flounder and sprat. Maximum Zn concentrations were higher in common shrimp from the present study than the EA study. The range of Pb, Cd, As and Cr concentrations measured in common shrimp in the present study and the range of Zn, Cu and As concentrations measured in sprat and flounder and Cd measured in sprat during the present study were far wider than the range given by the EA. This was not surprising due to the small sample size collected for the EA study compared to the considerable number of each species throughout the year for four years in this study. The metal concentrations presented by the EA are an average measurement for an amalgamation samples, whereas this study measured metal concentrations in individuals or amalgamations of two small individuals. Cd was not measured in the flounder by the EA and therefore could not be compared.

Table 4.9. Range of metal concentrations measured in common shrimp from the Hartlepool Power Station intake water in the Tees Estuary by the Environment Agency (1998-2002) and the present study (2000-2003).

	EA Study (mg/kg)	Present Study (mg/kg)
Zn	77.0 –112.0	87.7 – 168.6
Cu	36.7 – 49.9	32.9 – 53.0
Pb	2.1 - < 10	0.9 – 22.7
Cd	0.1 – 1.1	0.0 – 1.6
As	12.2 – 28.1	12.1 – 59.2
Cr	< 0.5 - < 10.0	0.1 – 4.7

N.B. The shrimp measured by EA were collected in August, whereas the shrimp in the present study were collected in July-August. 15+ shrimp were amalgamated in the EA study, whereas two shrimp were amalgamated in the present study with large individuals being sampled separately

Table 4.10. Range of metal concentrations measured in whole flounder and sprat from the Hartlepool Power Station intake water in the Tees Estuary by the EA (1998-2002) and the present study (2000-2003)

	EA Study (mg/kg)	Present Study (mg/kg)
Zinc		
Sprat	54.1 -147.0	5.2 – 294.6
Flounder	87.9 -99.3	23.0 – 257.8
Copper		
Sprat	2.8-12.1	1.7 – 34.3
Flounder	3.1 -6.6	1.1 – 10.8
Lead		
Sprat	0.7 -26.3	0.1 – 14.1
Flounder	2.0 -11.3	0.4 – 12.9
Cadmium		
Sprat	0.1 -1.0	0.0 – 3.3
Flounder	/	0.1 – 2.8
Arsenic		
Sprat	4.3 -14.9	0.7 - 57.0
Flounder	3.0 -9.2	0.7 – 29.3
Chromium		
Sprat	0.4 -63.2	0.2 - 3.6
Flounder	3.8	0.3 -3.6

N.B. The fish measured by EA were collected between May and September, whereas the fish in the present study were collected between May and October. Five flounder and 15+ sprat were amalgamated in the EA study, whereas individuals were sampled separately in the present study.

Copper concentrations in the common shrimp were comparable between the EA study and the present study, as were Pb concentrations in sprat and flounder. The maximum Cr concentrations given for sprat in the EA study were considerably higher than those given in the present study at 63.2 mg kg^{-1} dry mass, whereas the only Cr concentration given for flounder in the EA study was comparable with the highest concentration at 3.8 mg kg^{-1} dry mass. Murray and Portman (1984) reported that Cr concentrations in marine fish are generally less than 1 mg kg^{-1} wet mass, although higher mean Cr concentrations of 3.8 and 6.4 mg kg^{-1} wet mass were recorded in plaice and herring respectively from the Irish Sea. These concentrations were converted to dry mass to be comparable but were still considerably lower than the Cr concentration in sprat measured by the EA. The small sample of sprat used may have been an anomaly.

In this study, metal concentrations tended to be higher in Crustacea than in fish species, although there were exceptions of relatively low Zn concentrations in crabs and relatively high concentrations of Zn in both pleuronectid species and As and Cr in plaice. Cd concentrations were considerably higher in Crustacea than fish. Cd was reported to bioaccumulate in shore crab, common shrimp and prawns (Rainbow, 1998) and therefore would be expected to be high in Crustacea. Considerably higher Cu concentrations in Crustacea than fish were expected since Cu is a component of haemocyanin. Decapods regulate body concentration levels of Cu to within a narrow range, even when exposed to a wide range of ambient concentrations (Bryan, 1968). Decapods are detritus feeders and therefore take up sediment during feeding. Metal concentrations are bound to the particulate fraction of sediment and become elevated over time leading to increased bioavailability of metals to detritus feeders (Burgos and Rainbow, 1998). Concentrations of essential metals were expected to reflect the metabolic requirements of the different species (Dallinger and Rainbow, 1993).

There were significant interspecific differences in fish metal concentrations. Generally most metal concentrations were highest in pleuronectids, followed by clupeids and cod and were lowest in whiting. In contrast, the maximum Zn, Cu, Pb, As and Cr concentrations measured in flounder and sprat from the Tees Estuary by the Environment Agency, 1998-2002 were considerably higher in the sprat than the flounder (Table 4.10). Interspecific differences in metal concentrations may be related to habitat, fish mobility, diet or to other characteristic behaviour (Henry *et al*, 2004). Factors that may affect interspecific and intraspecific differences in metal concentrations are metabolism, body size, age, seasonality, salinity, pH and metal input into the Tees Estuary. Complex combinations of these different factors may mask trends in metal concentrations with species, body size or season.

In recent years heavy metal discharges to the waters of the Tees Estuary have been reduced, but high concentrations persist in the sediment from historic discharge (Davies *et al*, 1991; Huntley *et al*, 2002) so benthic organisms are most likely to be exposed to high metal

concentrations. Henry *et al* (2004) suggested that pleuronectids may be particularly affected by pollution because they reside and feed in bottom sediments where chemical contaminants accumulate. Crabs are also benthic species and contained high metal concentrations. The high metal concentrations are not consistent for all individuals and may be influenced by environmental and physiological factors. In an experiment by Berge and Brevik (1996) Zn, Cu and As concentrations did not exhibit significant increases in flounder tissues despite the sediment being severely contaminated, indicating that high metal contamination of sediment does not necessarily result in the high uptake of metal concentrations in benthic fish, at least not during a short-term period of one to three months. The PCA shows a clear difference in copper, lead, cadmium, arsenic and chromium concentrations between Crustacea and fish indicating that Crustacea are the main accumulators of these metals. The difference between metal concentrations in different fish species is less clear although whiting and cod are in a separate correlation grouping due to their low concentrations of Zn and plaice is grouped with common shrimp due to particularly high concentrations of As and Cr.

Exposure time in the estuary may cause variable metal content between and within species, with migratory species expected to have lower body burdens than residents, since metal levels tend to be higher in the estuary than offshore waters (Bryan and Langston, 1992). Species with migratory stages would be expected to have lower metal concentrations on returning from the open sea than after exposure time within the estuary. In a study of Hg contamination in components of the estuarine ecosystem, Elliott and Griffiths (1986) found higher concentrations in estuarine resident species (flounder, eelpout), compared with marine dermersal fish (cod, whiting) and the lowest concentrations in pelagic fish (sprat, herring etc). The high concentrations in the flounder are comparable with the present study but in most cases metal concentrations are higher in the clupeids than the gadids. Metal concentrations are also high in the plaice which primarily use the estuary as a nursing ground. The majority of plaice in this study were juveniles and hence had been exposed to metal concentrations in the Tees Estuary. Sprat and herring had relatively high metal concentrations in their body tissues compared to whiting in this study, although

concentrations were generally lower than in pleuronectids, despite their migratory nature and being fast swimming, pelagic, shoaling fish with a small body size and planktonic diet (Wheeler, 1969; Elliott and Dewailly, 1995). The relatively high metal levels may reflect their high fat content (Lawson *et al*, 1997), as some metals have an affinity with lipids.

Median Cu concentrations were higher in clupeids and saithe than in pleuronectids. Cu was the only metal studied by Henry *et al* (2004) where flatfish concentrations were relatively low compared to some roundfish. This contradicts the theory that Cu would be expected to be high in benthic organisms as it readily binds to sediments and organic matter. Cu may be so tightly bound to the sediment that it is not readily bioavailable. Cu concentrations may reflect intraspecific metabolic requirements rather than environmental levels due to being an essential metal. Concentrations of essential metals are expected to be regulated at optimal levels. The range of Zn concentrations in shore crab and swimming crab were similar to those in nine species of portunid crabs (Chan, 1990), but maximum Zn concentrations were higher. The range of Cu concentrations in shore crab and swimming crab were considerably lower than in nine species of portunid crabs (Chan, 1990). Eisler (1981) found that Zn concentrations in teleost fish from various locations ranged from 6 - 400 mg kg^{-1} dry mass. Zn concentrations in this study were far higher than the minimum value but considerably lower than the maximum values.

Lead levels in benthic organisms may be expected to be relatively high as Pb has a strong affinity with sediments and is sparingly soluble in seawater (Bryan and Langston, 1992). Organisms are not expected to have an advanced regulatory system for non-essential metals and so this metal was more likely to reflect sediment than an essential metal associated with the sediment, such as Cu. The uptake rate of Pb increased in tissues of edible crabs but was not significant in the flounder when exposed to contaminated sediments (Berge and Brevik, 1996). Pb concentrations in shore crabs and pleuronectids from the Tees Estuary were high compared to other fish species. Pb tends to accumulate in bone so may be expected to be low in small fish and Crustacea with relatively low bone content. Pb concentrations were low in all roundfish compared to pleuronectids in the Tees Estuary, irrespective of body

size but there were high Pb concentrations in the prawn, *P.elegans* and common shrimp, despite these being small and pelagic. Pb has been reported to bioaccumulate in *P.elegans* and common shrimp (Rainbow, 1988).

Cd concentrations were higher in pleuronectids from the Tees Estuary than in roundfish. Henry *et al* (2004) studied metal concentrations in flounder and plaice and found higher mean Cd content in these pleuronectids than in cod. Cod is a dermersal fish, with a diet of both pelagic and benthic invertebrates and fish and is less exposed to contaminated sediments than flatfish species, so may not be expected to bioaccumulate as much metal as benthic flatfish. Cadmium however, is more likely to be found in dissolved state in contrast to particulate metals such as Zn, Cu, Cr and Hg which remain bound to the organic particles whether suspended or in solution (Burgos and Rainbow, 1998). It may be expected therefore that pelagic fish will be exposed to relatively greater concentrations of Cd but the evidence contradicted this with higher Cd concentrations in pleuronectids than in roundfish.

Higher As concentrations have previously been reported in benthic organisms, such as crustaceans and flatfish than in roundfish and plankton feeders (Falconer *et al*, 1983). Estuarine sediments are a sink for dissolved inorganic As so concentrations might be expected to be higher in benthic organisms. This would explain the high concentrations in plaice and crabs and the lower concentrations in gadids but not the relatively high concentrations in shrimp and clupeids.

High concentrations of Cr in benthic species such as crabs and pleuronectids and low concentrations in more pelagic species indicate that high Cr concentrations are related to a benthic lifestyle rather than metabolic requirements. Cr concentrations are expected to be high in sediment because it tends to be associated with particulates, particularly those with small grain size and high organic and iron content sediments (Burt *et al*, 1992). The uptake rate of Cr increased in tissues of edible crabs but not flounder exposed to contaminated sediments (Berge and Brevik, 1996).

Metal uptake by fish occurs via the surrounding water and the diet, but there is confounding evidence in the literature about which one is the most important. The water environment would be expected to be an important route for metal uptake if the proportions of metals between solution and fish tissues were comparable. Cd and Hg concentrations were lower than other metals for all Tees Estuary species. This may reflect the low levels in the environment (Chapter 1). Viana *et al* (2005) stated that trace metal levels are commonly linked with the feeding habits of fish. Amundsen *et al* (1997) observed that species differences in heavy metal concentrations appeared to relate to the trophic status of the freshwater fish species, although Hg was the only metal where these species differences may have been due to biomagnification. Fish feeding on invertebrates had higher concentrations of Cd and Zn in their body tissues than piscivorous species. Estuarine fish species tend to consume high proportions of small epibenthic crustaceans, such as amphipods, shrimps, mysids and decapod crustaceans (Elliott and Hemingway, 2002). The low metal concentrations in gadids, which consume large numbers of fish as adults, compared to Crustacea and most other fish species studied, with the exception of large flounder, suggests that the metals do not biomagnify but rather decrease with trophic level. Metal concentrations were relatively low in whiting compared to the cod which are otherwise comparable in their dermersal lifestyle, migratory habits and body size (Wheeler, 1969; Elliott and Dewailly, 1995). Cod in the Humber estuary consumed high numbers of mysids, amphipods and decapod crustaceans, whereas whiting consumed higher numbers of mysids and amphipods, and large whiting consumed high proportion of decapod crustaceans (Marshall, 1995). Cod consumed more polychaetes than whiting. Cod also consumed smaller proportions of brachyuran crustaceans and fish, whereas these were only consumed in large proportions by large whiting. Cod consumed fish of a larger size than whiting. Cod in the Tees Estuary may also consume higher numbers of polychaetes than whiting and higher numbers of decapod crustaceans throughout their life than whiting and this may account for the higher burden of metals. Shore crab and common shrimp prey on infaunal populations of small bivalves, polychaetes and crustacean (Elliott and Hemingway, 2002). Common shrimp in the Humber estuary consume large numbers of small plaice (Marshall, 1995). High metal concentrations in shore crabs and common shrimp in the Tees

Estuary may have bioaccumulated from a diet with high proportions of smaller Crustacea and, in the common shrimp, of small plaice.

Metal concentrations in species may reflect different uptake methods (Rainbow, 1988). In accumulators metal concentrations will increase with age in tissues such as the kidney and hepatopancreas whereas in regulators the tissue concentrations of metals will correlate with those of the surrounding medium, unless levels exceed those that can be regulated. In studying food chain transference of metals, however, total body content in an accumulator may be misleading as detoxification can render the metals inaccessible to higher trophic levels (Dallinger and Rainbow, 1993). 60% of Zn in *Artemia salina*, for example, was found to be unavailable to young plaice (Milner, 1979).

Although metal concentrations tended to be higher in benthic pleuronectids than pelagic or benthopelagic fish, there were exceptions. In addition, metal concentrations tended to be higher in the pelagic common shrimp than the benthic, resident crabs. Metal concentrations may have been influenced by the age of the individuals analysed. Metal concentrations may decrease with age as they were diluted by growth or because adults regulate metals more efficiently (Clark, 1997). Alternatively, metal concentrations may increase with age as they accumulate over time. The gadids tended to have low metal concentrations and a relatively large body size in relation to most other organisms in the study, with the exception of some larger flounder and shore crabs. Although there were some gadids, flounder and shore crab individuals in this study that were large compared to other organisms studied they were relatively small compared to some fully grown individuals of these species. The high metal concentrations in the pleuronectids in this study may be influenced by the large proportion of juvenile pleuronectids in comparison to the roundfish species. Metal concentrations of Zn and Cu are often high in juveniles because they have high metabolic requirements for development (Mormede, 2001). Cu is essential for growth so young animals and neonates are normally richer in Cu than adults (Clark, 1997). A number of studies have reported that a greater quantity of Zn per body mass was required by rapidly growing juveniles and this was attributed to the relatively high metabolic rate requiring a high rate of enzyme reactions

(Cross *et al*, 1975; Pentreath, 1976; Badsha and Sainsbury, 1977; Milner, 1979). Non-essential metals may be high in juveniles because they have not been exposed for a sufficient time to develop tolerance (Mormede, 2001). Young animals tend to absorb a greater amount of Pb than adults and Cd has been observed to bioaccumulate with age. It has been demonstrated in a variety of organisms, including Crustacea and fish, that accumulation of non-essential metals during short-term exposure is most rapid in smaller individuals, whereas in the long-term non-essential metals are expected to bioaccumulate to a greater extent in older or larger individuals (Mormede, 2001). Cd concentrations would be expected to be higher in fish species with a large body size and higher trophic levels because Cd tends to be persistent with a proclivity to bioaccumulation and biomagnification (Dietz *et al*, 1996). This was not the case in this study. Cd concentrations were significantly higher in clupeids with small body sizes and consuming lower trophic level organisms, than whiting which have a higher trophic level and relatively high body size (Appendix K).

4.5.2. Variation of metal concentrations within and between species

Concentrations of essential metals are expected to vary less than concentrations of non-essential metals within and between species because intracellular concentrations of essential metal ions tend to be maintained at optimal levels by homeostatic mechanisms (Kress *et al*, 1999). Non-essential metals are expected to reflect environmental levels or to be influenced by biotic factors, such as age. Hg and Cd, for example, are expected to bioaccumulate with age and to biomagnify.

Zinc exhibited the lowest coefficient of variation of 0.14 or below for all species, as expected for an essential metal. There were interspecific differences between the coefficient of variation however, for Cu and Cr concentrations of between 0.17 for common shrimp to 0.4 for sprat and 0.03 in weever to 0.66 in herring, respectively. This may be influenced by seasonal variations in requirements or availability of these metals. In the non-essential metals, Pb concentrations also exhibited interspecific differences between the coefficient of variation of between 0.12 in crabs and 0.44 in sandeels. The relatively low variation in Pb for crabs, gadids, pleuronectids and weever may reflect the affinity of Pb for binding to

sediment and the metal may not be bioavailable (Bryan and Langston, 1992). Alternatively the low variation in Pb may reflect a tendency to accumulate in bone and to be stable once deposited in the bone (Mormede, 2001). The coefficient of variation for As varied from 0.22 in Crustacea to 0.43 in saithe. The coefficient of variation for Cd exhibited a range of values for different species from 0.25 in crabs to 0.76 in sprat. This may reflect the low ability to regulate this non-essential metal or a high variation in bioavailability.

Bi-monthly variation of metal concentrations within species was compared to investigate whether seasonal requirements or availability caused variations in metal concentrations. Variation of Zn continued to be lowest for all species over all seasons. This supports the view that Zn is well regulated. Zn, Cu and Cd concentrations in the shore crab increase in the autumn to winter months and decrease during the summer and may be influenced by ecdysis. Zn concentrations tend to be low in crab haemolymph during the early moult stage, and then increase significantly in the later stages of post moult (Chan, 1990). Ecdysis in British crabs principally occurs in the summer to early autumn (Crothers and Crothers, 1988). In addition, the seasonal difference in Cu variation in crabs may be explained by seasonal affects on the haemolymph, such as changes in body size due to Cu being an essential component in haemocyanin. Arsenic variation was higher in summer than winter and may reflect a fast accumulation of As during ecdysis or changes in body size.

In fish, there did appear to be a trend of relatively high Cu concentrations during the winter and relatively high Cr concentrations in the summer. The plaice, for example, had relatively high Cu concentrations in the summer. This trend was not statistically significant and did not occur for all species, so it may potentially have been due to sampling error. Seasonal variation in metal concentrations in fish but no clear pattern between species may indicate that each species has different seasonal intake and regulation.

4.5.3. Interactions between metal elements

Interactions between elements could potentially influence both the assimilation and the toxicity of metals (Amundsen *et al*, 1997). Correlation coefficients were used to assess whether there was any correlation between metal concentrations in Crustacea and fish species. There are a number of correlations between metals of less than 0.6 that are statistically significant. These are likely to be due to Type 2 errors because of the large sample size. Where strong correlations of greater than 0.6 were observed they were between Cd and Pb, Cu, As or Cr. This suggests Cd and Pb interact in common shrimp, shore crab, cod, sprat and herring, Cd and Cu interact in common shrimp, whiting, sprat and herring, Cd and As interact in common shrimp and Cd and Cr interact in shore crab. The interaction between metals may change with the concentration levels involved (Nugegoda and Rainbow, 1995; Rainbow *et al*, 2000). Individual variations, such as diet, lifestyle, sex and body condition, may mask interaction between the metals.

4.5.4. Seasonal variation in metal concentrations

Metal concentrations in the body tissues of biota reflect seasonal variation in growth rate, body condition, reproductive cycle, diet, water salinity, temperature and run-off from the land. In the Tees Estuary there was a summer maximum and winter minimum for a number of metal and species combinations, whereas metal concentrations were only statistically significantly higher in winter maxima than summer for Cu and As in whiting, As in sprat and Zn and As in herring.

Seasonal changes in growth rate can cause a different rate of change in metal concentrations but the metal content will remain constant (Phillips, 1980). Trace metal concentrations in aquatic biota may be diluted by fast growth and concentrated by slow growth. In temperate regions there is usually rapid growth in summer, diluting metals and slow growth in winter, concentrating metals. Most metal concentrations in the Tees Estuary increased in the summer therefore smaller individuals might be expected during the summer months. This was only the case for median dry mass of common shrimp and sprat. There was no obvious difference for other species, except the dry mass of herring was considerably higher in the summer. Since this was the only species with winter maxima of all metal concentrations

and statistically significant winter maxima for Zn and As, there does appear to be some affect of seasonal growth on metal concentrations.

Reproductive condition can significantly affect heavy metal concentrations in organisms in relation to seasonal maturation of gametes and fluctuations of biochemical components, body mass, water content and body condition. The timing of reproduction varies within species, depending on geographical location and water temperatures, so it is difficult to assess the affect of reproduction on seasonal metal concentrations. The reproductive condition of the adults analysed for metal concentrations in this study was not considered.

Bioavailability of free metal ions within the estuarine system varies significantly with seasonal differences in environmental variables, such as pH, salinity and temperature. Seasonal variation in metal concentrations are more likely to be exhibited by pelagic than benthic biota reflecting seasonality of metal uptake in solution, whilst concentrations in sediment are expected to be less influenced by seasonality and so relatively constant. The dominant uptake mechanism of each species, whether from solution, sediment or diet, is important in determining seasonal uptake of metals, as is their seasonal changes of lifestyle and diet. Seasonal availability of prey species may influence metal concentrations. Further study of seasonal diet of fish in the Tees Estuary is required to understand whether diet has a significant impact on seasonal variation in metal concentrations.

4.5.5. Variation of metal concentrations with body size

There were negative correlations between body size and some metal concentrations for common shrimp and the fish species. Large individuals tended to have relatively low metal concentrations and some small individuals exhibited higher metal concentrations. This general trend was confounded however, because there was a range of metal concentrations in small individuals. Higher metal concentrations in smaller, young fish have been observed in several studies (Cossa *et al*, 1992; Henry *et al*, 2004). Higher concentrations of metals in younger fish generally reflect the higher rate of metabolism compared to the older fish (Cossa *et al*, 1992). Alternatively, adults of some fish species, such as gadids, flounder and

herring, tend to feed on fish whereas the juveniles tend to feed on invertebrates, including Crustacea. Lower metal concentrations in the larger adults may reflect their diet, as metal concentrations were high in Crustacea in relation to fish.

Essential metals tend to be homeostatically controlled and are not expected to change with age, whereas non-essential metals are not well regulated and are expected to bioaccumulate with age (Thompson, 1990). In a number of studies, essential metals show little or no relationship with increasing body length of fish (Hornung and Ramelow, 1987; Thompson, 1990), although some studies report negative correlations between essential metals and body size. In this study there were significant negative correlations between dry mass and Zn concentrations in flounder, plaice, sprat and herring and between dry mass and Cu concentrations in whiting, flounder, sprat and herring. The significant negative correlation between concentrations of essential metals and fish length may be partly due to different adsorption rates across the gut or more efficient excretion in older fish (Amundsen *et al*, 1997). The demand for essential metals that are important constituents of enzymes or cofactors will be seasonal and may lead to a temporary difference in metal content in the body tissues.

The uptake rate of metals in crab larvae, small adults or newly moulted crabs may also be high in relation to the adult because of increased permeability of the exoskeleton due to reduced calcification and/or tanning (Rainbow, 1988). Juvenile crabs moult more frequently than adult crabs and so the body concentration of metals may have accumulated over the 'susceptible' stages in the crab lifestage (Chan, 1990). In this study however, there was no significant correlation between dry mass of shore crab and metal concentrations.

4.5.6. The affect of seasonal variation of body size on metal concentrations

The correlation between metal concentrations and body size may be confounded by seasonal changes. The influence of body size on seasonal changes in metal concentrations was considered. Correlations between body mass and metal concentrations for each season were assessed. Metal concentrations in a number of species may be diluted by higher body sizes in winter. Common shrimp, whiting and sprat had slightly higher dry mass, wet mass and length in winter. Herring had higher wet mass and length in summer than in winter and

winter maxima in metal concentrations. Body size may influence seasonal metal concentrations but be confounded by other physiological and environmental factors. Correlations were not strong enough to be able use regression equations to estimate metal concentrations in prey with changes in body mass. The influence of body mass on metal intake by predators could not be assessed and hence, median metal concentrations for each species were used to calculate metal uptake by predators in Chapter 6.

Metal concentrations-body size correlations may be confounded by age-related metabolism of metal concentrations, the opposing effects of ageing and tissue growth, the availability of the metal in the environment (Evans *et al*, 1993) or shifts in dietary and lifestyle habits of fish with age (Stronkhorst, 1992). Target organs or tissues for metal concentrations may change composition with growth or season. Seasonal metal concentrations may be influenced by different body composition, such as variation in lipids (Grimas *et al*, 1985). Lipid concentrations are expected to be high during richer food supply, such as in the summer months but lower after reproduction, also occurring in the summer months. Metal concentrations may also be influenced by seasonal variations in sub-cellular proteins or other molecules with an affinity for them (Phillips, 1980).

Some species of fish undergo large body mass changes due to spawning (Phillips, 1980). Whole body metal concentrations are likely to increase after spawning due to a combination of decrease in body mass and low metal content lost in the gametes. This supports the high metal concentrations observed in some species during the summer. Zn is an exception, however, as Zn content in the gonads is relatively high and so after spawning whole body concentration of Zn is likely to decrease. The only species exhibiting decreased summer Zn concentrations was the herring. This may have been influenced by spawning but as reproductive condition of the herring was not observed this can not be assessed. The smaller herring are not adults and hence will not be affected by spawning. Zinc concentrations increased in the summer for sprat and there was no significant seasonal difference in Zn concentrations for all other species.

4.5.7. Effects of mercury concentrations in Crustacea and fish from the Tees Estuary

The levels of Hg concentrations in Tees fish were mainly below detection levels of 1 mg kg^{-1} dry mass, with levels of 1-2 mg kg^{-1} dry mass being detected in only nine whiting. Concentrations below 1 mg kg^{-1} dry mass could not be detected by the apparatus used. The detection limit for Hg in this study is higher than the Environmental Quality Standard (EQS) for Hg in estuaries used in the EU Dangerous Substances Directive (Elliott and Hemingway, 2002) and therefore its value is limited. Hg concentrations were between 1 and 2 mg kg^{-1} dry mass in nine whiting however and these high concentrations indicate that further analysis of Hg concentrations is required in fish species in the Tees Estuary.

Bioaccumulation of Hg was observed to be stronger in slow-growing species (mainly predators) than fast-growing species (Mormede, 2001). Hg is expected to bioaccumulate and biomagnify in large, predatory fish (Eisler, 1981; Clark, 1997). As whiting body size increases they tend to switch to a predominately fish diet (Wheeler, 1969) this may explain why Hg was detected in nine whiting and no flounder Hg concentrations measured in whole flounder from the Tees Estuary by the EA, 1998 to 2002 and the muscle of flounder and whiting from the Tees Estuary by the EA, 1995 to 2002 ranged between 0.1 and 0.5 mg kg^{-1} dry mass. Mercury concentrations measured in fish for the present study were expected to be comparable or slightly lower than those measured in fish by the EA in the 1990s because Hg declined slightly in the surrounding water between 1990 and 1997, whilst concentrations in the sediment of the Tees Estuary remained relatively constant between 1992 and 2002, respectively (Table 1.1 and Figure 1.1 g). Mercury concentrations in the water and sediment of the Tees Estuary are low but Hg is toxic at low levels and it has the potential to bioaccumulate in prey and biomagnify through the food chain (Eisler, 1981).

Bryan and Langston (1992) and Elliott and Griffiths (1986) detected higher Hg concentrations in benthic species in contact with contaminated sediments than in pelagic fish feeding on plankton with low Hg content. Sediment appears to be the dominant uptake pathway for Hg in aquatic organisms, so higher Hg concentrations were expected in the

benthic flounder than the dermersal whiting in this study but this was not the case and Hg was only detected in whiting.

There is some evidence of Hg content being highly dependent on sex (Mormede, 2001). It appeared to accumulate faster in males than females, due to females shedding Hg within their eggs. The sex of the fish studied was not recorded and perhaps the nine whiting with detected Hg concentrations were male.

4.5.8. Limitations of the study

Salomons and Förstner (1984) suggested that, due to different toxicity and accumulation of pollutants between species, several species should be assessed as indicators of metallic contamination. A large sample size was required to account for variations in metal concentrations among individuals and between species, to test for seasonality and to encompass a range of body sizes. The number of samples collected per bi-monthly collection varied and so did the body sizes within species. This variation in body size influenced comparisons between metal concentrations and the influence could not be removed statistically due to the data being skewed and requiring non-parametric statistics.

Further knowledge of the ecology of the Crustacea and fish species in the Tees estuary, particularly regarding their diet and migratory movements, is not known. It would have been informative in assessing the difference between species and individuals. Analysis of stomach contents could be used to determine diet. The Crustacea and fish collected from the Hartlepool Power Station intake water however, were living in stressful conditions. Most individuals had empty stomachs and since they were enclosed in artificially high concentrations of species those that had food in their stomach may not necessarily haven eaten a typical meal. The cramped, stressful conditions and loss of body condition may have increased metal concentrations in body tissues, which would already be high in the small organisms that are taken in by the intake water since metal concentrations tend to be high in small organisms.

The sex of the fish may have had a significant influence on seasonal metal concentrations in reproductively mature fish but this was not recorded. There is some evidence of differences in metal behaviour and concentrations between male and female fish (Mormede, 2001). In addition, the timing of reproductive activity for each species in the Tees Estuary was unknown and spawning has been shown to influence metal concentrations in the biota.

Most studies compare metal concentrations in different body tissues rather than the whole organism so few comparisons with this study could be made. In addition, metal concentrations in the exoskeleton of Crustacea may not be assimilated by predators. Different metal concentrations between body tissues are therefore considered in Chapter 5.

4.5.9. Analytical problems with the Flame Atomic Absorption Spectrometry

Stripping Voltammetry is more sensitive in detecting metal concentrations than Flame Atomic Absorption Spectrometry (FAAS) but readings had a raised baseline due to noise from the high organic content and the results were too unreliable. There were a number of initial problems in obtaining readings for the metal concentrations using FAAS. The first FAAS used was an old machine and gave significantly lower results than those recorded on two newer models of FAAS. Different FAAS machines may give divergent metal concentrations despite regular servicing (Best, G., Huntsman Tioxide, pers. comm.). It is therefore important to use the same machine throughout the analysis. This is also an important consideration in comparing with other studies, particularly older studies when equipment would have been less technologically advanced.

The ICP machine used to measure Hg was not sensitive enough to detect the low concentrations of Hg in fish tissues. The machine was only able to detect Hg at 1 mg kg^{-1} and above. Hg is toxic at very low concentrations, so this detection limit was inadequate. It is not known whether the Hg concentrations in fish that could not be detected were extremely low or close to 1 mg kg^{-1}. With such a toxic metal these differences are extremely important, as are accurate, specific detection levels.

CHAPTER 5. COMPARTMENTALIZATION OF METALS IN CRUSTACEA AND FISH

5.1 INTRODUCTION

Metal concentrations in the whole body of Crustacea and fish were analysed in Chapter 4 because the purpose of the data is to allow a calculation of metal uptake by predators from prey species in Chapter 6. The exoskeleton of large crabs is not consumed by top predators such as seals and cormorants. The exoskeleton of smaller Crustacea may be consumed by predators but it is not known how much of the metal it contains will be absorbed (Rainbow, P., Department of Biology, Queen Mary and Westfield College, University of London, pers. comm.). Metal concentrations in the exoskeleton of Crustacea may not be assimilated by predators. Including the exoskeleton in the analysis of metal concentrations within the whole body of the crab will lead to an inaccurate calculation of the metal uptake by predators if metal concentrations are considerably different between the exoskeleton and the soft body of the crab. Total body metal load is the summation of the contents in tissues or organs (Dallinger and Rainbow, 1993). Each tissue can potentially contain metals in a metabolically available form or excess metals can be stored in a detoxified form to avoid toxic action. Organs acting as dump sites for detoxified metals accumulate high concentrations whilst other tissues involved in metal processing have lower, controlled concentrations. The bioavailability of metals to predators is dependent on the prey tissues ingested. This Chapter therefore measures the metal concentrations in the soft parts and the exoskeleton of Crustacea to assess whether the calculation of metal uptake by seals and cormorants consuming Crustacea could be adversely affected by including the exoskeleton in the metal analysis of the whole body.

Most studies of metal concentrations in fish measure concentrations in the muscle which is used for human consumption and the liver where metals tend to bioaccumulate. Whole body concentrations of fish measured in this study could not therefore be compared with metal concentrations measured in fish for the majority of other studies. Different metal concentrations between body tissues in flounder, *Platichthys flesus,* whiting, *Merlangius merlangus* and herring, *Clupea harengus* were therefore considered in this Chapter to allow

comparisons between metal concentrations measured in this study with those for similar fish species in other studies.

5.2 DIFFERENT METAL CONCENTRATIONS IN BODY TISSUES OF CRUSTACEA

The exoskeleton of large crabs is not consumed by top predators such as seals and cormorants and although the exoskeleton of smaller Crustacea may be consumed by these predators it is not known how much of the metal it contains will be absorbed. The exoskeleton is both a storage site and an excretion route for stored metals. The exoskeleton of shore crab stored 60-80% of zinc (Zn) from solution (Chan, 1990). The concentration of stored Zn increased as the total Zn load increased in crabs. The exoskeleton therefore serves as a sink for excess amounts of Zn in soft tissues and as a source when extra Zn is required. Metals are lost during moult, although metals may be resorbed at ecdysis (Rainbow, 1990). There is an initial increase in metal concentrations as moult exposes the permeable cuticle to the surrounding medium, at least until tanning and calcification are complete and this exposure leads to a temporary increase in the rate of metal uptake into the body (Furness and Rainbow, 1990). Zn and Cadmium (Cd) uptake rates in the glass prawn, *Palaemon elegans* increased immediately after moulting (Nugegoda and Rainbow, 1995). Cadmium uptake in crustaceans will change with the moult cycle and associated calcification of the exoskeleton because Cd and calcium have similar ionic radii and some Cd is taken up by energy-dependent routes for calcium uptake, depending on the activity of the calcium pump. Ecdysis and the frequency of the moult, therefore has a large influence on trace metal residues (Eisler, 1981). Shore crab, *Carcinus maenas* moults about 18 times during a 3 to 4 year life-span so a substantial amount of metal loss and uptake can occur via the exuvia.

The functions of the hepatopancreas include absorption and storage of nutrients, synthesis of digestive enzymes and detoxification of trace metals and xenobiotics (Rainbow, 1998). The major protein in haemolymph is haemocyanin and this plays an important role in transport and storage of metals, as well as the established role of oxygen transport. Approximately 85% of Zn and 90% of copper (Cu) in the haemolymph are bound to haemocyanin in crabs (Chan, 1990). The proportion of haemocyanin determines the changing levels of Zn and Cu in the blood. Haemocyanin structure is dynamic and affected by moult stage, diet, season, salinity, pH and

temperature. In the shore crab, haemocyanin concentrations decreased with moult (Rtal and Truchot, 1996). Nott and Mavin (1986) found that in the common shrimp, *Crangon crangon* the Cu released from haemocyanin breakdown during the moult was not lost but contributed to the formation and mobilization of Cu deposits in the hepatopancreas. Many crustaceans cease feeding during the moult so the decrease in blood haemocyanin and Cu concentrations may be a result of dilution from uptake of large volumes of water (Chan, 1990). Shore crab, for example, absorbed about 70% of water at ecdysis. Haemocyanin concentration is gradually increased during the inter-moult stage due to protein synthesis and reduction of blood volume due to increased tissue volume and growth. As a consequence Cu concentrations also increase at intermoult. Zn concentrations are lowest in crab haemolymph at the early moult stage, possibly as a response of dilution effects, then increase significantly in the later stages of post moult, possibly as a response to resorption of Zn bound in the exoskeleton. The estimated amount of Zn in the blood was about 12% of total body load (Chan, 1990). This high proportion in the blood suggests it may serve as a Zn storage site, as well as a transport medium.

Excretion of metals in Crustacea can occur through various routes with different relative importance, depending on the species. The efficiency of the excretion route influences bioaccumulation. Metals accumulated internally at higher concentrations than in the surrounding environment may be lost passively through permeable surfaces. An important excretion route in the glass prawn, the shore crab and the Dungeness crab, *Cancer magister* is across the gills (Bryan, 1968; White and Rainbow, 1984b; Rainbow, 1985, Rainbow, 1990). Metals may be excreted by defaecation with release of metal rich granules from the hepatopancreas or gut mucosa into the gut lumen of the alimentary tract. Shore crab, for example, releases lead (Pb) to the lumen in granules. Shore crab exposed to high concentrations of Zn over time excreted 40% in urine and 41% through the gills (Chan, 1990). The remaining 19% may be excreted through the gut mucosa or lost by desorption from the exoskeleton. The kinetics of Zn distribution and excretion in shore crab were different when the Zn was absorbed from food. Labeled Zn ingested was mostly transferred from the rest of the gut to the hepatopancreas for absorption. Over time, the percentage of Zn in the hepatopancreas decreased and increased in other tissues, thus indicating Zn redistribution. The highest dosage of Zn 12 days after the exposure was in the exoskeleton, indicating that the

exoskeleton plays an important role in Zn storage, although only about 40% of the 250 μg l^{-1} labeled Zn taken up from food was retained in the crabs. Approximately 10% was excreted with the faeces and the remaining 50% was expected to have been lost in soluble form via the gills or urine. Zn concentrations in different compartments are given in table 5.1.

Table 5.1. Compartmentalised Zn concentrations in Crustacea body parts (mg kg^{-1} wet mass) (Bryan, 1968) (/ = missing data)

Tissues	Shore crab	Common shrimp	Prawn, *Palaemon serratus*	Prawn, *Palaemon varians*
Exoskeleton	3.0	/	/	/
Blood	36.0	23.0	38.0	87.0
Hepatopancreas	56.0	78.0	64.0	65.0
Muscle	44.0	14.0	10.0	14.0
Gills	26.0	/	35.0	/
Excretory Organs	19.0	/	/	/

Copper in crabs is mainly utilized for the synthesis of haemocyanin and accumulates at high concentrations in the blood and hepatopancreas and low concentrations in the muscle (Eisler, 1981). The hepatopancreas is a major storage and regulation site for high Cu residues (Eisler, 1981).Cu comprised up to 93% of haemocyanin mass in the blood of the shore crab (Martin *et al*, 1977). Haemolymph Cu accounted for about 30% of the total Cu contents in 9 portunid crab species (Chan, 1990). Cu concentrations in different compartments are given in table 5.2.

Table 5.2. Compartmentalised Cu concentrations in Crustacea body parts (mg kg^{-1} wet mass) (Bryan, 1968) (/ = missing data)

Tissues	Shore crab	Common shrimp	Prawn, *Palaemon serratus*	Prawn, *Palaemon varians*
Exoskeleton	0.6	/	/	/
Blood	46.0	68.0	97.0	180.0
Hepatopancreas	42.0	520.0	185.0	137.0
Muscle	5.7	4.0	3.5	7.9
Gills	18.0	/	55.0	/
Excretory Organs	16.0	/	/	/

The main proportion of Pb in crustaceans is localised in the exoskeleton with low residues in other tissues (Eisler, 1981). Lead concentrations in crabs from Korea were 0.66 mg kg^{-1} and 0.50 mg kg^{-1} wet mass in exoskeleton and muscle, respectively (Won, 1973).

Eisler (1981) found the highest proportion of Cd in Crustacea to accumulate in the digestive glands, followed by the hepatopancreas or the kidney and to be lowest in the muscle. In a field experiment, Cd loaded pink shrimp, *Penaeus duorarum* contained Cd residues in the order of hepatopancreas> exoskeleton> muscle> serum (Nimmo *et al*, 1977). After seven days depuration in clean water, Cd in pink shrimp was significantly lower in the exoskeleton and the serum, unchanged in the hepatopancreas and increased in the muscle. A study of Cd uptake found that on entering the hemolymph the Cd was quickly displaced, some hemolymph was probably translocated to the hepatopancreas but a significant proportion of Cd was absorbed onto the gills and into the exoskeleton (Wright and Brewer, 1979)

Eisler (1981) found that arsenic (As) concentrated in lipid fractions primarily as organic As and this was thought to account for the lack of toxicity from relatively high body burdens. Arsenic concentrations from crabs in the field have been recorded as 37.8 mg kg^{-1} wet mass in the soft parts of *Cancer magister* (LeBlanc and Jackson, 1973), 6.1-6.4 mg kg^{-1} wet mass in crab muscle (Hoover *et al*, 1974), 3.7 mg kg^{-1} wet mass in crab muscle as total As and <0.5 mg kg^{-1} wet mass in crab muscle as inorganic As (Reinke *et al*, 1975).

Chromium (Cr) concentrations seldom exceed 0.3 mg kg^{-1} wet mass in muscle (Eisler, 1981). Concentrations measured in different tissues of the Atlantic rock crab, *Cancer irroratus* in the field did exceed these values (Greig *et al*, 1977) (Table 5.3).

Table 5.3. Compartmentalised Cr concentrations in Atlantic rock crab tissues (mg kg^{-1} wet mass) in the field (Greig *et al*, 1977).

Flesh	Digestive gland	Gills
<0.3-0.6	<0.5 – 1.2	0.8-2.5

5.3 DIFFERENT METAL CONCENTRATIONS IN BODY TISSUES OF FISH

Metal concentrations are generally considerably higher in fish liver than muscle (Andersen *et al*, 1973, Topping, 1973; Leatherland and Burton, 1974; Julshamn and Braekkan, 1975; Wharfe and Van Den Broek, 1977; Henry *et al*, 2004) (Tables 5.4 to 5.7). The differences in concentration are at least one order of magnitude and originate from differences in physiological functions of the muscle and liver. This is due to biotransformation of heavy metals in fish mainly occurring within the liver, followed by the kidneys, heart, plasma, intestine and brain and being lowest in the muscle (Marcovecchio *et al*, 1988; Huckle and Millburn, 1990). Zinc, Cu, Cd and Hg concentrations in four fish species from clean and polluted marine coastal sites in the Red Sea, Mediterranean Sea and North Sea were higher in the liver than the muscle (Kress *et al*, 1999). The enrichment factors ranged from 3 to 104, depending on species and location.

High metal concentrations in the liver correspond with elevated concentrations of metallothioneins in the liver of several fish species, including flounder (Brown, 1977). Individual and seasonal variation in fat content in the liver can occur (Grimas *et al*, 1985) and different metal distribution in body tissues may be influenced by this seasonal fat content as free metal ions such as Zn, Cu and Pb, are lipophilic ligands. Grimas *et al* (1985) found a negative correlation between Zn, Cu, Pb and Cd with increased fat content in cod liver. Liver has a high lipid fraction and consequently, it may be expected that the metal levels in the liver of fish will decrease with age as the fat content of the liver increases. Zn, however, tends to decrease with age in non-fatty tissues, the gills and muscle rather than the liver. The high lipid and metal content in the liver appears to contradict the evidence that metals are lipophobic. The liver may have segregated sites for metal and lipid storage or the metals may have been biotransformed and be in a lipophilic state. Metals which have been biotransformed, such as tetramethyl Pb and methylmercury, are non-polar and so can accumulate within fat rich tissues (Grimas *et al*, 1985).

Henry *et al* (2004) found that differences in Cu, Pb and Cd concentrations between field sites tended to be greater in muscle than liver (Table 5.5, 5.6 and 5.7). This suggests that muscle tissues tend to be more sensitive to surrounding environmental conditions, although

this sensitivity was variable with elements and fish species. Julshamn and Grahl-Nielsen (1996) used multivariate analysis to evaluate Zn, Cu, Pb, Cd, As and Hg concentrations in saithe, *Pollachius virens* and flounder muscle and liver. The difference between the two fish tissues tended to be greater than between the species with higher levels in the liver than in the muscle. This pattern is also evident in tables 5.4 to 5.7. Metal concentrations were however, generally higher in flounder liver than the saithe liver, particularly for Pb. The higher Pb concentrations in benthic flounder may have been a result of the strong affinity of Pb with the sediment and is sparingly soluble in seawater (Bryan and Langston, 1992). Copper and Cd uptake was higher in saithe muscle than flounder muscle. There was no difference in Zn uptake by muscle between the two species. This may have been due to Zn being an essential metal and hence, physiologically regulated so not expected to vary to any great extent within fish tissues with location or species (Kress *et al*, 1999). In this case however, Cu would not be expected to be higher in the saithe muscle.

Metal concentrations would be expected to be high in fish gill. The process of gas exchange requires seawater to passively flow over the large surface area of the gills and hydrophobic substances in solution in the seawater have a tendency to bind to the gill tissue. The gills are protected from heavy metals by a mucus secretion (McDonald and Wood, 1993). This secretion may be stimulated by exposure to heavy metals and can selectively bind metals such as Zn, Cu, Pb, Cd and mercury (Hg) thus retarding further diffusion.

5.4. METHODOLOGY

Fish and Crustacea were collected from Hartlepool Power Station cooling water intake for metal analysis of different body tissues (see chapter 4.2.). Ten common shrimps were collected in summer (June) and ten common shrimps were collected in winter (December) each year between 2000 and 2002. These common shrimps were dissected into soft body tissues and exoskeleton. Two samples of soft body tissues and exoskeleton were amalgamated to obtain 2 g of total sample. One hundred and eighteen shore crabs of between 16.2 to 162.2 g were collected between 2000 and 2002. Sixty-eight shore crabs were collected in the summer and 50 samples were collected in the winter. Each individual was dissected into exoskeleton, hepatopancreas, muscle and the remaining soft parts, including the blood (termed other parts). Each exoskeleton was measured individually, whilst samples of hepatopancreas, muscle and other parts were amalgamated from two to three crabs to obtain sufficient sample for analysis.

Thirty whiting, 30 flounder and 20 herring were collected. Fifteen samples of whiting and flounder and ten samples of herring were collected both in the summer (May-June) and in the winter (November-December). Each individual was dissected into liver, muscle and gill. All of the gill arch was analysed, comprising cartilage and gill lamellae. Two replicate samples of muscle were taken from each fish. These replicates were carefully homogenised to avoid different heavy metal loads in separate parts of the musculature. There was not sufficient material to take replicate samples of gill and liver, so one sample of gill and liver was analysed per fish. Other tissues, such as the kidney, did not provide sufficient tissue for AAS analysis.

The samples were dried to constant weight in the oven (at 60°C in order to avoid fat evaporation). Samples were digested and analysed using the method described in Sections 4.2.2. and 4.2.3. The body tissues of Crustacea were analysed for Zn, Cu, Pb, Cd, As and Cr concentrations and the body tissues of fish were analysed for Zn, Cu, Pb and Cd concentrations.

5.4.1. Statistical analysis

The Kolmo-gorov Smirnov test was used to test for normal distribution of metal concentrations in Crustacea and fish body tissues. Metal concentrations in Crustacea body tissues were not normally distributed so medians were compared and the non-parametric tests, Kruskal-Wallis H test and the Mann-Whitney U test, were used to test differences between metal concentrations in compartmentalised body tissues and between seasons.

Metal concentrations in fish body tissues were normally distributed. Means were compared and the two-way ANOVA was used to test for difference in metal concentrations between the three fish species, flounder, whiting and herring, and the three body tissues, muscle, liver and gill. The least significant difference (LSD) *post hoc* tests were used to discern which pairs of species and tissues were significantly different.

5.5. RESULTS OF HEAVY METAL PARTITIONING IN CRUSTACEA BODY TISSUES

5.5.1. Different metal concentrations in common shrimp tissues

Median metal concentrations were higher in the exoskeleton than the soft parts, except As concentrations which were higher in the soft parts (Table 5.4).

Table 5.4. Comparison of median and quartiles of metal concentrations in different body sections of common shrimp (mg kg^{-1} dry mass)

	Exoskeleton	Soft parts
Zn	141.8 (100.2-193.2) n=18	109.8 (96.7 -156.1) n=18
Cu	26.2 (16.9 -32.9) n=18	19.4 (16.5 - 28.2) n=18
Pb	8.6 (4.4 -11.8) n=18	3.0 (2.4 – 4.0) n=17
Cd	0.5 (0.2 -0.9) n=18	0.3 (0.2 -0.4) n=18
As	12.6 (4.3-17.6) n=18	18.5 (8.6-26.1) n=18
Cr	1.4 (0.7-2.6) n=18	0.7 (0.2-2.2) n=18

Mann-Whitney U tests were conducted to assess whether the differences between metal concentrations in the exoskeleton and the soft parts of common shrimp were significant. Pb (U =82, p<0.05) and Cd (U =94, p<0.05) concentrations in the exoskeleton were significantly higher than in the soft parts. There was no significant difference between exoskeleton and soft parts for other metals.

The concentration of a metal measures the amount of metals in a one mg sample of the whole organism, whereas the total metal content is the amount of metal in the whole organism. The metal concentration in the exoskeleton may be lower than in the soft parts but because the exoskeleton accounts for a higher proportion of the total body mass than the soft parts the total metal content may be higher in exoskeleton than the soft parts (Table 5.5). The intake of metal by the predator is determined by the amount of total metal content consumed.

Table 5.5. Dry body mass (median and quartiles) and average percentage contribution of exoskeleton and soft parts to the total body dry mass of common shrimp (n=18)

Total Dry Mass (g)	Exoskeleton (%)	Soft parts (%)
1.41 (1.05-1.90)	62%	38%

Metal concentrations in both the exoskeleton and soft parts were higher in the summer than in the winter (Table 5.6), although the sample size was small so results should be viewed with caution. Mann Whitney U tests showed significant seasonal differences in metal concentrations. For soft parts, Zn (U=18, p<0.05), Cd (U=9, p<0.01) and As (U=10, p<0.01) concentrations were significantly higher in the summer. For the exoskeleton, Zn (U =3, p<0.001), Cu (U =0, p<0.001), Pb (U=15, p<0.05), Cd (U=7, p<0.01) and As (U=8, p<0.01) concentrations were significantly higher in summer.

Table 5.6. Comparison of median and quartiles of summer and winter metal concentrations in the exoskeleton and soft parts of common shrimp (mg kg^{-1} dry mass)

	Exoskeleton		Soft parts	
	Summer	Winter	Summer	Winter
Zn	168.1 (147.9-272.8) n=10	96.3 (82.7-122.4) n=8	155.2 (100.0 -177.6) n=10	101.8 (94.6 -110.9) n=8
Cu	32.0 (27.3 -39.9) n=10	15.2 (8.7 -20.5) n=8	22.0 (18.5 - 28.2) n=10	16.0 (7.8 – 27.0) n=8
Pb	10.2 (7.7 -13.1) n=10	4.6 (1.9 -9.5) n=8	3.5 (2.5 – 9.7) n=9	2.6 (1.9 – 3.7) n=8
Cd	0.8 (0.5 -1.0) n=10	0.2 (0.1 -0.5) n=8	0.3 (0.2 -0.5) n=10	0.2 (0.1 -0.3) n=8
As	17.2 (12.2-21.7) n=10	4.6 (3.4-10.7) n=8	25.4 (19.3-28.1) n=10	8.8 (8.2-15.1) n=8
Cr	1.9 (1.1-4.2) n=10	0.8 (0.2-1.6) n=8	1.8 (0.3-3.4) n=10	0.5 (0.1-1.0) n=8

5.5.2. Different metal concentrations in shore crab tissues

The difference between metal concentrations in tissues of the shore crab were compared using boxplots (Appendix Q). There were significant differences in metal concentrations between body parts using the Kruskal-Wallis test ($p < 0.001$). Differences in metal concentrations between each pair of body tissues were determined using Mann-Whitney U test (Table 5.7).

Table 5.7. Significant differences in metal concentrations between each pair of shore crab body tissues from the Tees Estuary (Mann-Whitney U Test)

E = Exoskeleton, HP = Hepatopancreas, M = Muscle, OP = Other parts, NS = Non significant

Metals	Exoskeleton v HP	Exoskeleton v Muscle	Exoskeleton v other parts	HP v Muscle	HP v other parts	Muscle v other parts
Zn	< .001, HP	<0.001, M	<0.001, OP	<0.01, M	NS	<0.001, M
Cu	<0.001, HP	<0.001, M	<0.001, OP	<0.001, HP	<0.05, OP	<0.001, OP
Pb	<0.001, E	<0.001, E	<0.001, E	<0.01, HP	<0.001, OP	<0.001, OP
Cd	<0.001, E	<0.001, E	<0.001, E	<0.01, HP	NS	<0.01, OP
As	<0.001, HP	<0.001, M	<0.001, OP	<0.01,M	<0.01, OP	NS
Cr	<0.001, E	<0.001, E	<0.001, E	<0.001, HP	NS	<0.001, OP

The percentage of the total body content of metals may be higher in the exoskeleton despite low metal concentrations, since it accounts for a higher proportion of the total body mass. The proportions of mass for each compartmentalized body tissue were determined (Table 5.8).

Table 5.8. Total dry body mass (median and quartiles) and average % contribution of exoskeleton and soft parts to the total body dry mass of shore crab (n=241)

Total Dry Mass (g)	Exoskeleton (%)	Hepatopancreas (%)	Muscle (%)	Other (%)
12.87 (9.08-18.02)	69.08%	11.69%	10.03%	9.21%

The total metal content in all four body tissues was calculated and the percentage contributions of metals within each tissue were compared (Table 5.9).

Table 5.9. Total metal content in all four body tissues and the median percentage contributions (inter-quartile range in brackets) of different tissues in the shore crab.

	Zn	Cu	Pb	Cd	As	Cr
Total content (mg kg^{-1})	479.2	155.3	35.5	2.7	78.7	10.3
Exoskeleton (%)	8.9	5.8	43.9	44.6	7.6	40.2
	(8.5-9.2)	(5.3-5.9)	(43.3-44.4)	(44.3-44.9)	(7.3-8.0)	(39.7-40.3)
Hepatopancreas (%)	28.5	25.6	13.6	21.4	22.5	22.9
	(27.9-28.8)	(25.3-25.9)	(13.3-13.9)	(20.8-21.8)	(22.1-22.8)	(22.4-23.1)
Muscle (%)	35.7	15.9	9.3	15.1	36.7	12.2
	(35.2-35.9)	(15.4-16.2)	(9.0-9.6)	(14.7-15.5)	(36.2-37.0)	(11.9-12.3)
Other (%)	27.0	52.7	33.2	18.8	33.2	24.7
	(26.7-27.4)	(52.2-53.0)	(32.8-33.3)	(18.2-19.3)	(32.0-33.6)	(24.4-24.9)

The percentage of the total body content of metals ranked from highest to lowest in body tissues was:

Zn: Muscle>HP>Other>Exoskeleton.

Cu: Other>HP>Muscle>Exoskeleton.

Pb: Exoskeleton>Other>HP>Muscle

Cd: Exoskeleton>HP>Other>Muscle.

As: Muscle>Other>HP>Exoskeleton.

Cr: Exoskeleton>Other>HP>Muscle

Mann Whitney U tests showed that the only significant difference between metal concentrations in shore crab body tissues between winter and summer were significantly higher Cr concentrations in summer than in the winter (U=130, p<0.05). Metal concentrations may change in crabs due to growth. The wet mass of the exoskeleton and other parts were significantly higher in the summer (U =1284, p<0.05) and (U=127.5, p<0.05), respectively.

5.6. RESULTS OF HEAVY METAL PARTITIONING IN FISH BODY TISSUES

Zinc, Cu, Pb and Cd were analysed in muscle, liver and gill for three fish species. The results are presented as means and standard deviations (Table 5.10).

Table 5.10. Comparison of mean metal concentrations in different body sections of fish (mean and standard deviations in mg kg^{-1} dry mass)

a) Zn

	No.	Muscle	Liver	Gill
Whiting	30	27.5 +/- 3.9	95.5 +/- 21.4	109.6 +/- 13.5
Flounder	30	51.7 +/- 14.6	169.0 +/- 17.9	165.8 +/- 42.0
Herring	20	44.8 +/- 9.8	74.4 +/- 14.4	104.2 +/- 41.1

b) Cu

	No.	Muscle	Liver	Gill
Whiting	30	2.4 +/- 1.1	11.5 +/- 2.1	10.0 +/- 2.3
Flounder	30	3.6 +/- 0.8	25.3 +/- 4.3	9.3 +/- 1.9
Herring	20	4.9 +/- 0.9	23.1 +/- 7.3	10.4 +/- 3.7

c) Pb

	No.	Muscle	Liver	Gill
Whiting	30	3.4 +/- 0.9	7.1 +/- 1.3	11.0 +/- 1.4
Flounder	30	3.7 +/- 0.8	8.7 +/- 1.7	9.3 +/- 1.9
Herring	20	4.4 +/- 2.0	10.7 +/- 3.2	15.0 +/- 3.0

d) Cd

	No.	Muscle	Liver	Gill
Whiting	30	0.4 +/- 0.1	0.7 +/- 0.2	0.6 +/- 0.2
Flounder	30	0.4 +/- 0.1	0.8 +/- 0.3	0.6 +/- 0.2
Herring	20	0.4 +/- 0.3	0.9 +/- 0.6	1.1 +/- 0.3

All metal concentrations were lowest in muscle. Cu concentrations were highest in the liver of all species, whereas Zn, Pb and Cd concentrations were high in the gills or the liver, depending on the species.

A two-way ANOVA was conducted on each metal to determine whether there was a difference in concentrations between species and between tissues (Table 5.11). There was a highly significant difference between species and tissues for all four metals.

Table 5.11. Results of two-way ANOVA to test for difference in metal concentrations between the three species and between the three tissues

	Tissues	Species
Zn (mg kg^{-1})	F = 268.66, df = 2, p<0.001	F = 167.06, df = 2, p<0.001
Cu (mg kg^{-1})	F = 532.78, df = 2, p<0.001	F = 66.15, df = 2, p<0.001
Pb (mg kg^{-1})	F = 460.90, df = 2, p<0.001	F = 47.58, df = 2, p<0.001
Cd (mg kg^{-1})	F = 54.84, df = 2, p<0.001	F = 14.85, df = 2, p<0.001

Post Hoc Tests were conducted to assess which variables exhibited significant difference (Table 5.12 and Table 5.13).

Table 5.12. Post-Hoc Tests (LSD) to assess significant difference of metal concentrations between the three species

Metal	Species	Herring	Whiting
Zn	**Flounder**	***, Fl	***, Fl
	Herring		***, Herr
Cu	**Flounder**	NS	***, Fl
	Herring		***, Herr
Pb	**Flounder**	***, Herr	**, Fl
	Herring		***, Herr
Cd	**Flounder**	***, Herr	NS
	Herring		***, Herr

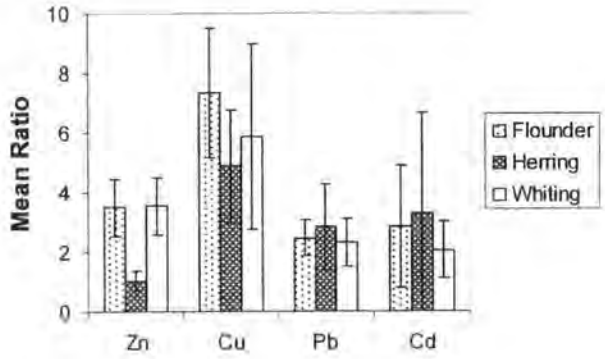
Table 5.13. Post-Hoc Tests (LSD) to assess significant difference of metal concentrations between the three body tissues

Metal	Tissue	Liver	Gills
Zn	**Muscle**	***, liver	***, gill
	Liver		***, gill
Cu	**Muscle**	***, liver	***, gill
	Liver		***, liver
Pb	**Muscle**	***, liver	***, gill
	Liver		***, gill
Cd	**Muscle**	***, liver	***, gill
	Liver		NS

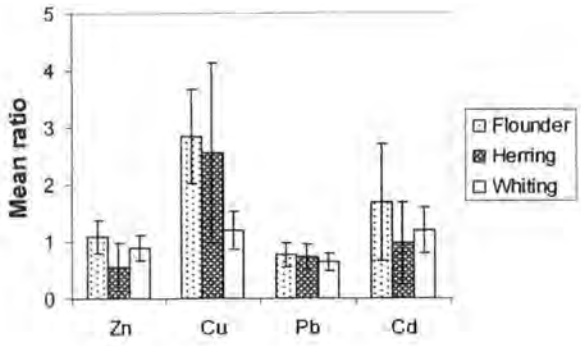
Metal concentrations were significantly higher in the flounder and herring than in the whiting. Metal concentrations were significantly higher concentrations in gill and liver than in muscle. Differences in metal concentrations between liver and muscle (L/M), liver and

gill (L/G) and gill and muscle (G/M) were calculated using ratios (Figure 5.1). The differences between ratios indicate that metal concentrations in tissues and relative distribution between tissues depend on both trace metal properties and fish species.

a)



b)



c)

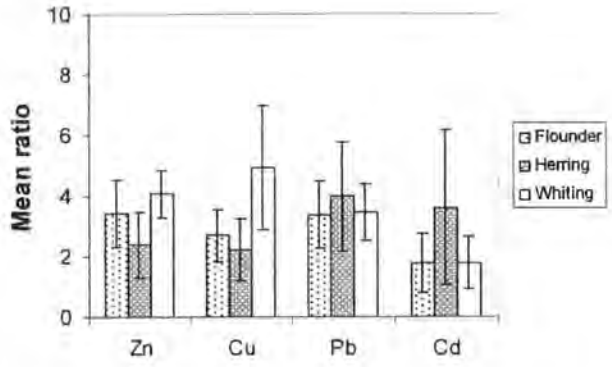


Figure 5.1. Mean ratios and standard deviations between body tissues for flounder, herring and whiting a) liver:muscle ratio b) liver:gill ratio c) gill:muscle ratio

5.7. DISCUSSION OF HEAVY METAL PARTITIONING IN FISH AND CRUSTACEA BODY TISSUES

5.7.1. Partitioning of heavy metals in body tissues of Crustacea

It may be assumed that the exoskeleton should be excluded from total pollutant intake by predators as they do not consume the exoskeleton of large crabs and the exoskeleton of small crabs and shrimps tends to pass through the digestive system as a whole or in large fragments. The accuracy of this process is debatable however, as the exoskeleton is exposed to digestive attack within the predators stomach so metal digestion may occur during passage through the gut (Rainbow, P., Department of Biology, Queen Mary and Westfield College, University of London, pers. comm.). Tissue specific partitioning was conducted to assess the relative distributions of metals between exoskeleton and soft tissues.

In common shrimp, median metal concentrations were higher in the exoskeleton than the soft parts for all metals, except As concentrations which were higher in the soft parts than the exoskeleton. The difference between metal concentrations in the exoskeleton and the soft parts was only statistically significant for Pb and Cd concentrations. Higher concentrations in the exoskeleton may be an indication of relatively high levels in the surrounding medium, since the exoskeleton is both a storage site and an excretion route for stored metals (Rainbow, 1990). If the metal concentrations stored in the exoskeleton are not ingested by the predator then the uptake of metal concentrations from common shrimp will be less than those quoted in Chapter 4.

In shore crab, Zn, Cu and As concentrations were lower in the exoskeleton than other body tissues, whereas Pb, Cd and Cr concentrations were highest in the exoskeleton. This may be due to Zn, Cu and As being required for metabolic purposes, whereas Pb and Cd are non-essential metals and so stored in the exoskeleton. Cr is an essential metal but external concentrations may be higher than required for metabolism and therefore stored in the exoskeleton. It is possible that the low Zn, Cu and As content in the exoskeleton of Tees Estuary crabs was a result of moult. Metals are lost during moult, although a portion of the metal may be resorbed at ecdysis (Rainbow, 1990).

The distribution of Zn in the shore crab from the Tees Estuary was muscle>hepatopancreas>other parts>exoskeleton. In contrast, the Zn distribution pattern in a study by Chan (1990) was muscle > exoskeleton> hepatopancreas> blood> gills> other parts> hypodermis when crabs were exposed to high concentrations of 3162 $\mu g\ l^{-1}$ and exoskeleton> muscle> hepatopancreas> blood> other parts> gills> hypodermis when crabs were exposed to low levels of Zn of 23 $\mu g\ l^{-1}$ and 100 $\mu g\ l^{-1}$. The exoskeleton of each of the crab species had the lowest Zn and Cu concentrations of all the tissues but it accounted for 50 to 75 % of the total dry mass of the crab, so constituted 8.86% and 5.76% of the total body load in shore crab, respectively. Depledge (1989) reported Zn content in the exoskeleton of only 4% of the total body load, whereas Rainbow (1985) reported Zn and Cu contents in the exoskeleton of 59% and 55% of the total body load, respectively. In the study by Chan (1990) there was a greater proportion of Zn in the exoskeleton of shore crab from the polluted Restronguet Creek (about 60%) than in crabs from less polluted areas (about 45%). This indicates that the exoskeleton plays an increasingly important role in Zn accumulation when crabs are exposed to high Zn concentrations, suggesting that crabs in the Tees Estuary had not recently been exposed to high Zn concentrations.

Zinc and Cu concentrations in the hepatopancreas of Tees estuary shore crabs were lower than observed by Bryan (1976). Zn in the muscle of shore crabs from the Tees Estuary contained 36% of the total body load, which was lower than reported in the study by Chan (1990) where muscle in the legs contained about 50% of the total body Zn of crabs. This may be due to Zn playing a specific role in muscle contraction. Cu in the muscle of shore crabs from the Tees Estuary contained 35% of the total body load which was considerably higher than the 10 to 20% contained in the muscle of shore crabs studied by Chan (1990). The other parts of Tees Estuary shore crabs contained about 27 % and 53 % of Zn and Cu, respectively. In contrast, Cu and Zn levels in the hypodermis, gills and the remaining tissues of shore crab in the study by Chan (1990) represented less than 10% of the total body metal loads. The low concentrations in shore crab tissues reported by Chan (1990) may indicate a relatively fast rate of metal flux across the tissues or relatively low metal permeability. Alternatively, the different partitioning of metals between body parts in the two studies could be the result of different external concentrations.

Eisler (1981) found the main proportion of Pb in crustaceans to be localised in the exoskeleton with low residues in other tissues. This was corroborated in this study of metal concentrations in Crustacea from the Tees Estuary, with high Pb concentrations in the exoskeleton of common shrimp and shore crab. Cd concentrations in common shrimp and shore crab from the Tees Estuary were higher in the exoskeleton than the soft parts. The proportion of Cd concentrations in the exoskeleton of Crustacea decreased after depuration in clean water (Won, 1973; Nimmo *et al*, 1977; Wright, 1977). High concentrations measured in the exoskeleton of common shrimp and shore crab may indicate high Cd concentrations in the ambient environment either stored in the exoskeleton or adsorbed on the external surface.

Periodic uptake and excretion of metals may be reflected in transfer of metals between the crab tissues, according to the tissues being metabolically active or storage sites. There were no obvious seasonal transfer pathways, however, that were consistent for all metals. Tissues have metabolic requirements for essential metals and hence are not expected to fluctuate as much as non-essential metals. The only metal that was significantly different seasonally was Cr, despite it being an essential metal. This indicates seasonal variation in metabolic requirements of Cr, more fluctuation in seasonal bioavailability of Cr than other metals in either the environment or the diet or variation in the uptake rate of Cr with changes in salinity and temperature. The wet mass of the exoskeleton and the other parts was significantly greater in summer exoskeleton than winter but the difference was not significant for the dry mass. This suggests that water content may be high in the summer and cause dilution of metal concentrations. The only significant difference in seasonal metal concentrations however, was higher Cr concentrations in the summer. Moult may also account for seasonal variation of Cr concentrations in the exoskeleton (Rainbow, 1990).

Metals can be passively adsorbed onto binding sites on the exterior of the invertebrate, particularly the exoskeleton. The relatively low metal concentrations compared to those quoted in the study by Chan (1990) may indicate that either external concentrations in the Tees Estuary were lower than those in crabs exposed in the experiments by Chan (1990). If the metal concentrations stored in the exoskeleton are not ingested by the predator then the

uptake of Pb and Cr concentrations from shore crab will be less than those quoted in Chapter 4.

5.7.2. Partitioning in body tissues of fish

In this study, the majority of fish consumed by seals and cormorants were expected to be eaten whole and so predator metal intake was represented by whole body metal concentrations (Chapter 4). Rae (1968) suggested that seals may not eat the heads of large fish but the fish consumed by the Tees seals were not large so the whole fish was analysed rather than just the body. In addition, Hall *et al* (1998) found that large otoliths were recovered from faecal samples providing evidence that seals do consume the heads of large prey. Tissue specific partitioning can however, aid in our understanding of pollutant uptake by fish. It allows comparison of metal concentrations for fish between several studies in different locations and time periods. A number of studies have shown tissue specific partitioning of metal concentrations in fish (Andersen *et al*, 1973, Topping, 1973; Leatherland and Burton, 1974; Julshamn and Braekkan, 1975; Wharfe and Van Den Broek, 1977; Henry *et al*, 2004).

Mean muscle Zn concentrations were generally less than 10 mg kg^{-1} wet mass (Thompson, 1990). Zn concentrations in the muscle of whiting from the Lower Medway Estuary, Kent were less than 10 mg kg^{-1} wet mass but Zn concentrations in the muscle of benthic flounder and plaice from the Lower Medway Estuary (Wharfe and Van Den Broek, 1977) and flounder from the Tees Estuary were higher (Table 5.14). Zn concentrations in the Tees Estuary were divided by 5 to give an estimated metal concentration expressed as wet mass to allow comparisons with Zn concentrations measured in the Lower Medway Estuary. Zn concentrations in whiting and flounder liver the Tees Estuary were slightly lower than in the Lower Medway Estuary, whereas Zn concentrations in flounder muscle were similar. Higher Zn concentrations may be expected in the Lower Medway Estuary since the study had taken place during the 1970's when high metal concentrations were disposed of in estuaries. Table 5.14 also shows higher metal concentrations in the liver than the muscle, with particularly high concentrations in the flounder.

Table 5.14. Comparison of Zn concentrations (mg kg^{-1} wet mass) in liver and muscle of fish species from coastal waters of the Lower Medway Estuary, Kent (Wharfe and Van Den Broek, 1977) and Zn concentrations (mg kg^{-1} dry mass/5) in liver and muscle of fish species from the Tees Estuary, 2000-2003

	Muscle		Liver	
Species	**Lower Medway Estuary**	**Tees Estuary**	**Lower Medway Estuary**	**Tees Estuary**
Whiting	9.1-9.2	4.3-7.0	28.3	12.2-26.4
Flounder	1.2–18.2	6.0-15.6	53.8-68.9	24.7-37.9
Plaice, *Pleuronectes platessa*	10.0-11.9	/	38.9	/

Muscle Cu levels generally have means of around 0.3 to 0.8 mg kg^{-1} wet mass (Thompson, 1990). Cu concentrations in the muscle of fish species from the North Sea were less than 10 mg kg^{-1} wet mass (roughly 50 mg kg^{-1} dry mass) (Table 5.15). Copper concentrations are higher the Tees Estuary than other locations in herring muscle, flounder muscle and whiting muscle and liver. Copper concentrations are lower in the flounder liver than in some other locations in the North Sea. These higher metal concentrations in the Tees Estuary have occurred despite some of the other studies having taken place during the 1970's when high metal concentrations were deposited into estuaries. Cu concentrations were higher in the liver than the muscle of all fish species but particularly high in the flounder (Table 5.15).

Table 5.15. Mean concentrations +/- standard deviations or inter-quartile range of Cu (mg kg^{-1}) in liver and muscle of fish species from coastal waters of the North Sea (dry mass unless otherwise stated) a) Andersen *et al*, 1973 b) Julshamn and Braekkan, 1975, c) OSPAR (Oslo and Paris Comission)Quality Status Report 2000, d)Henry *et al*, 2004 e) Present study (2000-2003) (/ denotes missing data)

Species	Location	Muscle	Liver	Ref.
Herring	Inner Oslofjord	4.4	/	a
	Tees Estuary	4.9 +/- 0.9	23.1 +/- 7.3	e
Sprat, *Sprattus sprattus*	Inner Oslofjord	5.6	/	a
Cod, *Gadus morhua*	North Sea	0.6	5.8	b
	North Sea	/	10.0	c
	Iceland	/	0.5 – 5.0	c
	Norway	/	0.4 – 0.6	c
	Calais, N. France	1.2 +/- 0.7	9.1 +/- 3.8	d
	Boulogne, N.France	1.6 +/- 1.1	8.6 +/- 4.3	d
	Bay of Somme, N.France	0.9 +/- 0.1	9.5 +/- 2.5	d
Whiting	Tees Estuary	2.4 +/- 1.1	11.5 +/- 2.1	e
Flounder	North Sea	/	0.3 – 50.0	c
	Iceland	/	8.0 – 11.0	c
	Dunkirk, N.France	0.78 +/- 0.04	52.2 +/- 25.1	d
	Calais, N. France	1.8 +/- 0.4	49.6 +/- 7.6	d
	Boulogne, N.France	1.6 +/- 0.9	25.6 +/- 22.8	d
	Bay of Somme, N.France	0.92 +/- 0.10	33.0 +/- 12.8	d
	Bay of Seine, N.France	1.2 +/- 0.2	34.0 +/- 23.4	d
	Tees Estuary	3.6 +/- 0.8	25.3 +/- 4.2	e
Plaice	Dunkirk, N.France	1.2 +/- 1.0	11.1 +/- 10.8	d
	Calais, N. France	2.2 +/- 2.3	9.1 +/- 3.5	d
	Boulogne, N.France	1.2 +/- 0.7	11.7 +/- 11.4	d
	Bay of Somme, N.France	1.1 +/- 0.2	11.3 +/- 3.4	d

Lead concentrations in the muscle of marine fish tend to be very low, generally less than 1 mg kg^{-1} wet mass (roughly 5 mg kg^{-1} dry mass) and values rarely exceed 2 mg kg^{-1} wet mass (roughly 10 mg kg^{-1} dry mass) (Thompson, 1990). Pb concentrations in the muscle of fish species from the North Sea were generally less than 1 mg kg^{-1} wet mass (roughly 5 mg kg^{-1} dry mass) (Table 5.16), except in the muscle of sprat and herring in the Inner Oslofjord (Andersen *et al*, 1973). Lead concentrations in whiting muscle and liver from the Tees Estuary were higher than those in cod from other locations and Pb concentrations in flounder muscle and liver from the Tees Estuary were higher than those in flounder and plaice from other locations in the North Sea. Hardisty *et al* (1974a) found Pb concentrations in the muscle to

be less than 20% of those in the heart, liver or kidneys. This was not the case for all species of fish from the North Sea but Pb concentrations were higher in the liver than the muscle of all the fish species (Table 5.16).

Table 5.16. Mean concentrations +/- standard deviations of Pb (mg kg^{-1} dry mass) in liver and muscle of fish species from coastal waters of the North Sea (a) Andersen *et al*, 1973 b) Julshamn and Braekkan, 1975 c) Henry *et al*, 2004) d) Present study (2000-2003) (/ denotes missing data)

Species	Location	Muscle	Liver	Ref.
Herring	Inner Oslofjord	7.0	/	a
	Tees Estuary	4.4 +/- 2.0	10.7 +/- 3.2	d
Sprat	Inner Oslofjord	8.2	/	a
Cod	North Sea	0.09	0.43	b
	Calais, N. France	0.01 +/- 0.02	ND	c
	Boulogne, N.France	0.07 +/- 0.04	ND	c
	Bay of Somme, N.France	0.001 +/- 0.001	0.21 +/- 0.07	c
Whiting	Tees Estuary	3.4 +/- 0.9	7.1 +/- 1.3	d
Flounder	Dunkirk, N.France	0.02 +/- 0.02	0.09 +/- 0.01	c
	Calais, N. France	0.04 +/- 0.05	0.08	c
	Boulogne, N.France	0.04 +/- 0.04	0.26 +/- 0.02	c
	Bay of Somme, N.France	0.008 +/- 0.021	0.16 +/- 0.07	c
	Bay of Seine, N.France	0.05 +/- 0.03	0.19 +/- 0.18	c
	Tees Estuary	3.7 +/- 0.8	8.7 +/- 1.7	d
Plaice	Dunkirk, N.France	0.07 +/- 0.07	0.09 +/- 0.07	c
	Calais, N. France	0.10 +/- 0.19	0.32 +/- 0.41	c
	Boulogne, N.France	0.03 +/- 0.03	0.16 +/- 0.13	c
	Bay of Somme, N.France	0.01 +/- 0.03	0.38 +/- 0.28	c

Cadmium concentrations in fish tend to be low, often below detection limits with values rarely exceeding 0.2 mg kg^{-1} wet mass in muscle tissue (roughly 1 mg kg^{-1} dry mass) (Thompson, 1990) This was the case for Cd concentrations in the muscle of fish species from the North Sea (Table 5.17). Cadmium concentrations in whiting muscle and liver from the Tees Estuary were higher than those in cod from other locations and Cd concentrations in flounder muscle and liver from the Tees Estuary were higher than those in flounder and plaice from other locations in the North Sea. The upper quartile Cd concentration of flounder from Iceland was higher than concentrations in flounder from the North Sea. Cd concentrations were higher in liver than muscle for all fish species.

Table 5.17. Mean concentrations +/- standard deviations or interquartile range of Cd (mg kg^{-1}) in liver and muscle of fish species from coastal waters of the North Sea (dry mass unless otherwise stated) a) Topping, 1973 b) Julshamn and Braekkan, 1975 c) Leatherland and Burton, 1974 d) OSPAR (Oslo and Paris Comission)Quality Status Report 2000, e) Henry *et al*, 2004 f) Present study (2000-2003) (/ denotes missing data)

Species	Location	Muscle	Liver	Ref.
Herring	Scottish Waters	0.03-0.12 (Wet Wt.)	/	a
	Tees Estuary	0.4 +/- 0.3	0.9 +/- 0.6	f
Cod	North Sea	0.02	0.09	b
	North Sea	/	0.08 - 0.1	d
	Iceland	/	0.02 - 0.1	d
	Norway	/	0.08 - 0.1	d
	Calais, N. France	0.01+/-0.01	0.08 +/- 0.06	e
	Boulogne, N.France	0.01+/-0.01	0.08 +/- 0.04	e
	Bay of Somme, N.France	0.004+/-0.001	0.05 +/- 0.04	e
Whiting	Tees Estuary	0.4 +/- 0.1	0.7 +/- 0.2	f
Flounder	Solent Region	0.03	/	c
	North Sea	/	0.7 – 0.8	d
	Iceland	/	0.5 - 3.0	d
	Dunkirk, N.France	0.003 +/- 0.001	0.26 +/- 0.13	e
	Calais, N. France	0.02 +/- 0.01	0.42 +/- 0.15	e
	Boulogne, N.France	0.01 +/- 0.01	0.61 +/- 0.73	e
	Bay of Somme, N.France	0.006 +/- 0.003	0.52 +/- 0.21	e
	Bay of Seine, N.France	0.02 +/- 0.02	0.20 +/- 0.07	e
	Tees Estuary	0.4 +/- 0.1	0.8 +/- 0.3	f
Plaice	Dunkirk, N.France	0.007 +/- 0.020	0.12 +/- 0.09	e
	Calais, N. France	0.03 +/- 0.04	0.23 +/- 0.28	e
	Boulogne, N.France	0.01 +/- 0.01	0.27 +/- 0.16	e
	Bay of Somme, N.France	0.007 +/- 0.003	0.42 +/- 0.18	e

Zinc, Cu and Pb concentrations in whiting muscle and flounder top eyed fillet can also be compared between this study and a study conducted by the Environment Agency in the Tees Estuary, 1998-2002 (Table 5.18). Metal concentrations have been measured in these fish tissues by the EA since 1992 but earlier concentrations were expressed as wet mass. This makes comparision difficult as dry mass can be roughly estimated from wet mass but accuracy would be reduced. The range of Cu concentrations measured in whiting and flounder were comparable between both studies, whereas the maximum Zn and Pb

concentrations measured in flounder and whiting by the EA were higher than in this study. Metal concentrations were expected to be slightly lower in the present study than the EA study as these Zn and Cu declined slightly in the water body of the Tees Estuary since 1997, whilst Pb and Cd concentrations remained relatively constant (Chapter 1, Table 1.1) and Zn, Cu and Pb declined slightly in the sediment of the Tees Estuary since 1992 (Chapter 1, Figure 1.1 a-c). Only Pb was measured exactly in flounder by the EA and concentrations were not considerably different. Comparable concentrations were expected since the samples for both studies were collected from the Hartlepool intake water. The present study reports the range of metal concentrations in muscle between May and September, whereas the fish muscle was collected in August by the EA. The metal concentrations presented by the EA are an average measurement for an amalgamation of five fish per sample; whereas this study measured metal concentrations two replicate samples of muscle per individual.

Table 5.18. Range of metal concentrations measured in the muscle of whiting and flounder from the Tees Estuary by the Environment Agency (1998-2002) and the present study (2000-2003)

Metal	Species	EA Study (mg/kg)	Present Study (mg/kg)
Zn	Whiting	36.0 –63.0	21.5 -33.9
	Flounder	40.0 –105.0	30.0 – 76.3
Cu	Whiting	2.4 - < 5.0	1.1 – 3.7
	Flounder	1.2 - < 7.0	2.5 – 5.0
Pb	Whiting	1.1 - < 10.0	2.2 – 4.6
	Flounder	1.2 – 11.3	2.0 -5.0

N.B. The fish measured by the EA were collected in August, whereas the fish in the present study were collected in May-September. Five fish were amalgamated in the EA study, whereas two replicates of muscle were taken from each individual fish in the present study and the mean concentration reported.

Studies of metal concentrations in fish tend to analyse the fish muscle because it is most commonly consumed by humans, whereas the liver may be analysed because it tends to accumulate metals (Usero *et al*, 2003). This is due to biotransformation of metals in fish mainly occurring within the liver (Marcovecchio *et al*, 1988; Huckle and Millburn, 1990) and a correlation between high metal concentrations and a high content of metal binding MTs in the liver (Brown, 1977). The liver is hence, a good indicator of chronic exposure to

heavy metals. Metal concentrations were also measured in fish gills in this study. They are expected to be high since gas exchange requires seawater to passively flow over the large surface area of the gills and hydrophobic substances in solution in the seawater have a tendency to bind to the gill tissue. The gills are protected from heavy metals by a mucus secretion (McDonald and Wood, 1993). This secretion may be stimulated by exposure to heavy metals and can selectively bind metals such as Zn, Cu, Pb, Cd and Hg thus retarding further diffusion.

Metal concentrations appeared to vary less between species than the distribution between tissues (Andersen *et al*, 1973, Topping, 1973; Leatherland and Burton, 1974; Julshamn and Braekkan, 1975; Wharfe and Van Den Broek, 1977; Henry *et al*, 2004). This pattern was reflected in this study with lower concentrations in muscle than in liver and gills for all metals and all species. The high concentration of metals in the gills are likely to reflect the concentrations in surrounding water as they will tend to bind to the gill tissue whilst they passively flow over the gill surface area with the ambient water. The high Pb concentrations in gill compared to other tissues may be a result of Pb tending to occur in high concentrations in bone and skin.

Tissue specific partitioning rather than species specific partitioning is expected for essential metals due to physiological regulation (Thompson, 1990). High concentrations of essential metals are expected in the liver, due to retention by metallothioneins. The low concentration of Zn and Cu in the muscle is likely to be a reflection of low levels of metallothionein in muscle and, hence low levels of bound metal. Zn concentrations however, were significantly different between all three species with highest concentrations in the flounder. Higher concentrations in the liver of flounder compared to gadids and clupeids also occurred in other studies (Wharfe and Van Den Broek, 1977; Julshamn and Grahl-Nielson, 1996), whereas Zn concentrations in flounder muscle were not significantly different to that in saithe muscle (Julshamn and Grahl-Nielson, 1996). Zn concentrations in flounder muscle in the Lower Medway Estuary, Kent ranged from low to high concentrations, 1.2-18.2 mg kg^{-1} dry mass (Wharfe and Van Den Broek, 1977). This high concentration of Zn in benthic flounder may indicate higher concentrations in the sediment or food. Cu concentrations were significantly higher in flounder and herring than whiting. A

tendency for higher Cu concentrations in the liver of pleuronectids than in the liver of gadids and clupeids has been documented (Andersen *et al*, 1973; Julshamn and Braekkan, 1975; Wharfe and Van Den Broek, 1977; Henry *et al*, 2004). Cu concentrations in muscle appeared to be low in all species with little difference between species, possibly indicating metabolic regulation of this essential metal.

Lead and Cd concentrations are lower in benthic flounder and demersal whiting than in pelagic herring suggesting that the main source is from solution rather than food. A tendency for higher Pb concentrations in the muscle of clupeids than in the muscle of gadids and pleuronectids has been documented (Andersen *et al*, 1973; Julshamn and Braekkan, 1975; Henry *et al*, 2004). No comparable concentrations of Pb in clupeid liver were found. Cd concentrations in other studies appeared to reflect location rather than species (Havre *et al*, 1973; Topping, 1973; Leatherland and Burton, 1974; Julshamn and Braekkan, 1975; Pentreath, 1977; Henry *et al*, 2004). This may explain why there was no significant difference between Cd concentrations in flounder and whiting.

Metal concentrations in flounder liver were compared between the present study and the highest median concentrations measured in fish in Scottish and English waters by the National Monitoring Programme Survey of the Quality of UK Coastal Waters (MPMMG, 1998) (Table 5.19). The raw data was not available from the other studies so statistical analysis of the differences between metal concentration. Metal concentrations measured in fish in Scottish and English waters by the MPMMG, 1998 were reported as wet mass, whereas in this study metal concentrations measured in fish were reported as dry mass. To allow comparisons the data from this study was divided by 5 to give a rough estimate of the metal concentration expressed as wet mass in fish. The highest median concentrations of Pb measured in the liver of fish from Scottish and English waters were recorded in dab, *Limanda limanda* liver off the Tay Estuary. This figure was comparable to the median Pb concentration in flounder liver from the Tees Estuary, suggesting that Pb concentrations were high compared with other UK coastal areas. The highest median concentrations of Cd measured in the liver of fish from Scottish and English waters were also recorded in dab liver off the Tay Estuary, whilst the highest median concentrations of Cd recorded in English waters were in dab liver offshore of the Humber and

the Tyne Estuaries. These figures were considerably higher than the median and maximum Cd concentrations in the liver of flounder from the Tees Estuary. This suggests that Cd concentrations were relatively low in the Tees Estuary compared to the most polluted UK coastal areas located to the south and north of the Tees Estuary. Dab are migratory and spend less time in estuaries than flounder. Metal concentrations would therefore be expected to be higher in flounder liver therefore suggesting that metal concentrations in the Tees Estuaries are comparable or lower than in other industrial estuaries.

Table 5.19. Comparison of metal concentrations in flounder from the Tees Estuary (1999-2002) compared with the highest reported median concentrations in fish measured during the MPMMG survey of the UK (1998)

Metal	MPMMG, 1998 Highest reported median values in fish from the UK (mg/kg wet mass)	Tees Estuary (1999-2002) Median and range in flounder (mg/kg dry mass/5)
Lead	0.62 mg/kg in dab liver, off the Tay	0.65 (0.40-1.37) mg/kg in flounder liver, Tees Estuary
Cadmium	0.8 mg/kg in fish liver, off the Tay 0.37 mg/kg in fish liver, offshore of Tyne-Humber	0.07 (0.02-0.11) mg/kg in flounder liver, Tees Estuary
Arsenic	~ 20 mg/kg in dab muscle, off the Tees and Forth ~6mg/kg, flounder muscle	1.58 (0.96-2.79) mg/kg in whole flounder, Tees Estuary

5.7.3. Comparison of metal concentrations in soft parts of Crustacea and whole fish.

The high metal concentrations measured in crustacean compared to fish may be partly due to the metal content in the exoskeleton. The exoskeleton contributes a mean of 62% of the body mass of common shrimp and a mean of 69% of the body mass of shore crabs. High metal concentrations in the exoskeleton will therefore have a significant effect on the overall body burden of Crustacea and the high metal concentrations quoted in Chapter 4 may be an over-estimate of the concentrations of metals assimilated by predators. Not including metal concentrations stored in the exoskeleton of small crabs and shrimp that were consumed by

seals and cormorants may lead however, to an under-estimate of metal concentrations assimilated by predators as they pass through the digestive system. It is therefore important to ascertain whether metals are digested from the exoskeleton by the predator.

Further work is required to determine whether the high metal concentrations are on the exterior or incorporated within the exoskeleton. This will greatly affect the amount of metal available to the predator. The metal concentrations of ingested and non-ingested exoskeletons could be measured and the difference calculated to understand whether Crustacea do assimilate significant concentrations of metals from the exoskeleton. It may then be possible to compile an equation that would account for the proportion of metal uptake from the exoskeleton. The metal intake by predators from the consumption of prey species is estimated in Chapter 6. The calculation assumes that predators' take in the whole body content of metals.

CHAPTER 6. HEAVY METALS IN HARBOUR SEALS FROM THE TEES ESTUARY

This chapter investigates the uptake of heavy metals via the diet by the top predator from the Tees Estuary, the harbour seal, *Phoca vitulina*. Metal intake from prey is estimated from the seasonal biomass of prey species consumed, calculated in Chapter 3, and the seasonal metal concentrations in these prey species, calculated in Chapter 4.

Metal intake from the diet was compared to metal concentrations in the body organs of a four year old, male grey seal recovered from Seal Sands in August, 2000 and a 2 year old, female harbour seal recovered from Greatham Creek in January, 2003. Metal body burdens in the two seals were estimated and compared with metal intake from the diet. Metal output in seal faecal samples was compared with metal intake to estimate retention in seals. This retention estimate was then compared with metal concentrations in the seal tissues.

6.1. EFFECTS OF HEAVY METALS ON HARBOUR SEALS

Many contaminants including several heavy metals accumulate in large, long-lived marine mammals (Laws, 1995). Seals exhibit a range of physiological and biochemical adaptations for living in oceans and deep diving including large storage compartments for blood and wide amplitudes of seasonal cycles in fat storage and mobilization. These adaptations may influence the susceptibility or resistance of seals to toxic substances (O'Hara and O'Shea, 2001). The high fat storage means that accumulation of lipophilic contaminants is particularly high.

Some trace metals, such as Cu and Zn, are essential for the health and growth of marine mammals and they have developed mechanisms to regulate the internal concentrations of these essential trace elements (Law, 1995; Bustamante *et al,* 2004). Law *et al* (1991, 1992) measured Zn concentrations in the liver of marine mammals (seals, porpoises and dolphins) collected from waters around the British Isles. Zn concentrations were between 20 to 100 mg kg^{-1} wet weight and the authors therefore postulated that 20 to 100 mg kg^{-1} wet weight was approximately the range at which Zn was maintained by marine mammals and therefore levels above 100 mg kg^{-1} wet weight were likely to cause a failure in the regulation mechanism. Law

et al (1991) also found that Cu concentrations in the livers of adult marine mammals tended to be within the range of 3 to 30 mg kg^{-1} wet weight and probably represents the normal range of homeostatic control in marine mammals. Law *et al* (1992) measured Zn and Cu levels in the livers of grey seals, common seals and harbour porpoise carcasses. Thirty-nine individuals had levels exceeding either 30 mg kg^{-1} of Cu or 100 mg kg^{-1} of Zn but only one individual harbour porpoise, *Phocoena phocoena* stranded on the Isle of Man, exceeded values of both metals simultaneously. Eisler (1981) stated that the maximum concentration of Cu recorded in the liver of harbour seals, *Phoca vitulina* was 194 mg kg^{-1} dry mass in a pup that was born prematurely. Concentrations of Zn and Cu elements could have toxic effects in the event of the control mechanism becoming overloaded and being unable to maintain homeostasis.

Arsenic has been reported in numerous species of marine mammals but at concentrations and/or forms not considered toxic (O'Shea, 1999). Organoarsenics, including arsenobetaine, are relatively non-toxic and can be eliminated via the kidneys in mammals. Arsenobetaine is generally the major As species present in marine tertiary consumers, including the harp seal, *Phoca groenlandica* and the ringed seal, *Phoca hispida* (Kubota *et al*, 2002). The major As compounds in the livers of the pinnipeds (harp seal and ringed seal) were arsenobetaine, arsenocholine, dimethylarsinic acid, methylarsonic acid and an unidentified As compound.

The main heavy metals of concern in marine mammals are the non-essential metals; Hg, Cd and Pb. These non-essential metals can be toxic even at low concentrations and may interfere with essential elements (O'Hara and O'Shea, 2001). Alternatively, these non-essential metals may be distributed within cellular compartments and rendered inert. Non-essential metals are not expected to be as well regulated as essential metals and this is reflected by reported extensive variation in total Hg and Cd levels in pinnipeds both inter- and intra-specifically. Fish eating species, including harbour seals and grey seals, *Halichoerus grypus* tend to exhibit relatively high Hg concentrations compared to those feeding on benthic invertebrates, such as the walrus, *Odobenus rosmarus* (Roberts *et al*, 1976; Drescher *et al*, 1977; Caines, 1978; Van de Ven *et al*, 1979; McKie *et al*, 1980; Reijnders, 1980). This assessment of biomagnification of Hg is complicated by the tendency for Hg concentration to increase with age in pinniped

tissue (Koeman *et al*, 1973; Roberts *et al*, 1976; Drescher *et al*, 1977; Reijnders, 1980; Dehn *et al*, 2005). Geography is also likely to affect Hg concentrations.

Law (1996) reported no differences in Hg concentrations between male and female seals, whereas the concentration of Hg was higher in the male than in the female seal in a study by Gaskin *et al* (1972). Watanabe *et al* (1998) detected significantly decreased tissue levels of both Hg and Cd in adult male Baikal seals compared to females. This was thought to be a result of different feeding rates between male and females. There was no difference in Cd concentrations in tissues between the sexes (Honda *et al*, 1983).

Relatively high Hg levels in some prey species are natural and pinnipeds exposed to these high dietary levels have evolved adaptations to demethylate the toxic, organic form of Hg into the less toxic, inorganic storage form in the liver (Reijnders, 1980). Up to 90% of the Hg in pinniped food is present as the highly toxic methylHg, whereas only 10-15% is found in their body tissues (Reijnders, 1980). Organic Hg in Californian sea lions, *Zalophus californianus,* predominates in the muscle tissue (Buhler *et al*, 1975). Animals weakened by disease have been observed to accumulate higher levels of methylHg suggesting that they may be unable to detoxify organic Hg as efficiently as healthy animals (Dietz *et al*, 1990). MethylHg poisoning arises from a very high intake rate accumulating in the body faster than the detoxification and mineralization process can facilitate. This high threshold required will be approximately in the range of 0.25 to 25 mg kg^{-1} body mass per day in seals. Most data on Hg in marine mammals is based on total or inorganic Hg, due to the expense and time required to quantify concentrations of the methylated form (O'Hara and O'Shea, 2001).

A correlation between Hg and selenium has been detected in marine mammals. The atomic ratio of Hg: selenium was close to 1 in the livers of wild grey seals from the UK (Van de Ven *et al.*, 1979) and the livers of various species of pinnipeds and cetaceans from other regions (Koeman *et al*, 1975). This equimolar Hg: selenium ratio is not apparent in fish species that make up a significant portion of marine mammal diet, nor in birds and other mammals. Selenium is thought to counteract the toxicity of Hg by immobilizing it as inert mercuric selenide. The protective capacity of selenium is likely to be limited however, at higher concentrations when Hg, selenium or both compounds may exert a hazardous body burden. Hg

and selenium concentrations were determined in samples of liver, kidney and brain in seal species off the coast of Norway (Skaare *et al*, 1994). They detected variable levels in different species. Two coastal species, the harbour seal and grey seal, had tissue concentrations 10 to 40 times higher than the two arctic species, the harp seal and the ringed seal. The highest concentrations were found in the grey seal.

A number of studies have been conducted to determine whether metal concentrations bioaccumulate in seals with age (Hepplestone and French, 1973; Sergeant and Armstrong, 1973; Koeman *et al*, 1975; Roberts *et al*, 1976; Drescher *et al*, 1977; Watanabe *et al*, 1998; Bustamante *et al*, 2004; Dehn *et al*, 2005). Zinc and Cu concentrations were higher in juveniles and subadaults than adults in the liver, kidney and muscle of sixty Baikal seals, *Phoca sibirica* (Watanabe *et al*, 1998). The decrease of concentrations with age was particularly rapid during the immature stages. There was no correlation between age and tissue levels of Zn and Cu in the harbour seal from German North Sea coast (Drescher *et al*, 1977). In contrast, Cd in liver tissue of harbour seals from German North Sea coast (Drescher *et al*, 1977), Hg in seal liver tissue (Hepplestone and French, 1973; Sergeant and Armstrong, 1973; Koeman *et al*, 1975; Drescher *et al*, 1977) and Pb in liver and kidney tissue of harbour seals from German North Sea coast (Drescher *et al*, 1977) increased with age. Cd concentrations in the kidney and liver were low in harbour seals from off the coasts of East Anglia and West Scotland but they accumulated with age (Roberts *et al*, 1976). Cd concentration has been shown to continue to increase with age in several pinniped species (Roberts *et al*, 1976; Drescher *et al*, 1977). Increases in Cd concentrations with age are often in association with correlated increases in Zn concentrations but these increases in Zn are not statistically significant. Bustamante *et al* (2004) reported continuous accumulation of Cd with increasing age in grey seals. In contrast, there was a continuous but gradual increase of renal Cd in ringed seals from Canada up to a peak age of around 10, followed by a decline with increasing age (Dehn *et al*, 2005). This suggested that physiological changes associated with aging in the kidney can lead to a decrease in Cd content. Arsenic concentrations may increase with age and body size due to higher uptake rates from prey in relation to excretion rates (Kubota *et al*, 2001). This is especially the case for young animals with a higher feeding rate.

Mercury can biomagnify in food chains, particularly in its methylated form, which is most toxic (Law, 1995). The trophic supply of Hg increases progressively with the mammal's growth, as the amount of food eaten and the preferred prey size increase (André *et al*, 1991). Thus the body burden of Hg in marine mammals would be expected to increase with age as has been reported in harbour seals (Reijnders, 1980; Miles *et al*, 1992) and grey seals from Eastern Canada (Sergeant and Armstrong, 1973). Although total Hg levels increase in seal liver with age, the proportion of methylHg typically decreases with age (O'Shea, 1999).

6.1.1. Tissue distribution of metal concentrations in harbour seals

Heavy metal storage and detoxification tend to be organ specific and metal dependent (Dehn *et al*, 2006). Oehme (1978) states that metals in marine mammals should be found in the highest concentrations in the liver and kidney because these organs have roles in detoxification, filtering and excretion of substances and so are sites of sequestration. Heavy metals are rendered metabolically unavailable by metallothionein (MT) and positive correlations between MT content and metal concentrations were observed in grey seal liver and kidney, particularly for Zn and Cd (Teigen *et al*, 1999). High Cd and, to a lesser degree, Cu concentrations in the liver and kidney of the striped dolphin, *Stenella coeruleoalba,* were related to MT, irrespective of growth and sexual stages, whereas MT was not significantly correlated to Pb concentrations (André *et al*, 1991). In contrast, the concentration of MTs, and hence the ability to sequester metals, in harbour seals has been correlated with age (O'Hara and O'Shea, 2001). In the grey seal, MT levels were significantly higher in fetuses than in adults and particularly higher than in juveniles (Teigen *et al*, 1999). The low level of MT in juveniles may be due to dilution as a result of fast growth. Zn concentrations in the liver and kidney were found to be high in juveniles compared to levels in adults and fetuses but Cd concentrations in juveniles tend to be low probably due to growth dilution and MT reduction later in pregnancy, causing either significant metal transfer to other tissues, excretion or both.

In the striped dolphin, metal concentrations tend to be high in the liver, kidney, bone and skin and low in the brain and blubber (André *et al*, 1991). Exceptions were relatively low Zn and Pb concentrations in the liver and high concentrations of Pb in the blubber and especially in the bone. The distribution of metal concentrations in blubber and different integration during growth stages may be due to the composition of the blubber i.e. the protein, lipids and blood

content. The low concentrations of metals in the brain, particularly Cd and Pb, are regarded as being due to the 'blood-brain' barrier (André *et al*, 1991).

Drescher *et al* (1977) reported higher levels of Zn, Pb and Hg in the liver of harbour seals, followed by levels in the kidneys and the lowest levels were detected in the brain. Some studies, however have found Zn concentrations to be as high or higher in the kidney as the liver (Hamanaka *et al*, 1982). In a study of Zn, Cu, Cd, Hg and Se concentrations in grey seals from the Faroe Islands, Northeast Atlantic Ocean, the highest concentrations of Zn, Cu and Hg were found in the liver, whereas the kidney contained the highest Cd concentrations (Bustamante *et al*, 2004). Zinc concentrations in harbour seals were consistently higher in the liver than the kidney, although there was overlap of minimum liver concentrations and maximum kidney concentrations (Thompson, 1990). Copper concentrations were higher in the liver than in the kidney, muscle and brain (Eisler, 1981; Thompson, 1990). Liver Zn and Cu concentrations were higher in fetuses than adult harbour seals (Thompson, 1990).

Lead levels in pinniped tissues are generally low, with concentrations rarely exceeding 1 mg kg^{-1} wet mass in any tissue (Thompson, 1990). Lead concentrations in the liver of harbour seals from the east coast of England and Scotland however, ranged from between 3 to 12 mg kg^{-1} wet mass. In a study by Law *et al* (1992) concentrations of Pb in the liver of marine mammals in Britain (seals, porpoises and dolphins) were measured as between 0.05 to 7.0 mg kg^{-1} wet mass with generally low Pb concentrations (<1 mg kg^{-1}), except where marine mammals inhabited industrialised coastal regions. Grey seals and cetaceans inhabiting Liverpool Bay, North West England had elevated liver concentrations of Pb up to 4.3 mg kg^{-1} (Law *et al*, 1992).

Tissue distribution of Cd in pinnipeds tends to decrease in the order kidney>liver>muscle (Thompson, 1990). This pattern of metal distribution is supported by higher Cd concentrations in the kidney of seals in coastal waters of East Anglia and West Scotland. In the Dutch Wadden Sea and German coastal waters however, maximum Cd concentrations in the liver were higher than those in the kidney. Cadmium concentrations were consistently higher in the kidney and liver than in the blubber and muscle (Eisler, 1981; Wagemann and Muir, 1984). High Cd

concentrations of up to 600 mg kg^{-1} dry mass have been reported in the kidneys of pinniped seals (O'Hara and O'Shea, 2001). Ideally Cd concentrations should be investigated in both the liver and the kidney although the kidney is a better measure of background exposure to Cd because Cd is extremely stable in the liver. Cd levels in the liver may correlate with selenium levels suggesting a degree of protection by selenium from Cd toxicity (Magos and Webb, 1980).

Liver concentrations of arsenic in the harbour seal were 0.2-1.7 mg kg^{-1} wet mass (Koeman *et al*, 1973). Chromium concentrations measured in seal tissues were high in the blubber and low in the brain, kidney and placenta. Hexavalent Cr is more readily assimilated than the trivalent state and at least 100 times more toxic, with high accumulation in the red blood cells (Foster, 1963). Highly vascularized organs in harbour seals, found dead on collection did not however, contain as high a concentration of Cr as the brain.

Generally, the concentration of total Hg is highest in the liver, intermediate in the muscle and lowest in the blubber (Eisler, 1981). Total Hg in the liver can be stored at very high concentrations due to an apparent capacity to detoxify and store Hg. The liver is therefore generally the most important accumulator of Hg in pinnipeds (Wagemann and Muir, 1984), followed by kidney levels. Most studies concentrate on Hg concentrations in the liver. The maximum concentrations in the liver reported, include 751 mg kg^{-1} wet mass in harbour seals (Reijnders, 1980) and 1097 mg kg^{-1} wet mass in grey seals (Simmonds *et al*, 1993). In addition a high proportion of methylHg is generally found in the muscle tissue of marine mammals (Dietz *et al*, 1990). The liver, skeletal muscle and blubber contained 95% of the Hg burden from 18 tissues and organs analyzed in the striped dolphin (André *et al*, 1991). The concentration of total Hg increased slightly with age in both the muscle and liver and the concentration of organic Hg increased in the liver, which suggests a low rate of excretion for this metal even at low concentrations.

Mercury contamination of seals from a UK study appeared to reflect the known inputs into coastal waters (Simmonds *et al*, 1993). Mercury concentrations in grey seals from the Dee Estuary were ten times higher than those in Norfolk Wash harbour seals, with liver-Hg

concentrations amongst the highest ever reported (mean of 571 mg kg^{-1} wet mass). Norfolk Wash harbour seals were some ten times more polluted than harbour seals from northeast Scotland. The primary source of Hg is dietary so prey from the Mersey River and Dee Estuary were likely to have been the source of contaminants in the grey seals. The Dee Estuary receives contemporary anthropogenic inputs from urban and industrial catchments (Turner, 2000), notable amongst these are a gas fired power station, three separate paper mills, a chemical manufacturing plant, numerous smaller manufacturing industries and two sewage treatment works. The high contaminant levels in Dee Estuary grey seals have been correlated with uterine blockages (Baker, 1989). The harbour seals in the study were mostly pups however, whereas the grey seals were adults and since burdens tend to increase with age this may have accentuated the difference in concentrations.

Zinc accumulates in skin and hair in relation to pigmentation (André *et al*, 1991). In several freshwater and marine fish species and the striped dolphin the Zn content was two to four times higher in black skin than in white skin. In the striped dolphin the concentration of Zn in the grey skin was intermediate between the levels in black and white skin. This might also be the case for seals. Copper accumulates in skin in relation to pigmentation (André *et al*, 1991). Relatively higher Pb concentrations have been detected in the skin and bone of marine mammals compared to their soft organs (Law, 1995). Nearly 90% of the total body burden of Pb in marine mammals was found in the bones. Wenzel *et al* (1993) reported that Pb levels were below the detection level in most skin samples, in contrast to the high Pb concentrations reported in the skin by Law (1995). Clark (1997) suggested that Pb does not significantly bioaccumulate in the soft tissues of seals. In one study on Californian sea lions Pb accumulated in hard tissues, bone and teeth, in significantly higher concentrations than in soft tissues such as fat and muscle (Braham, 1973). This was also the case in the harbour seal (Roberts *et al*, 1976). Metal concentrations in the bone tend to reflect calcium content suggesting that heavy metals accumulate in the bone with ossification (André *et al*, 1991). Cadmium levels were below the detection level in most skin samples of harbour seals (Wenzel *et al*, 1993).

6.2. METAL INTAKE AND OUTPUT ROUTES IN HARBOUR SEALS

The tissue burden of contaminants, including heavy metals in top predators reflects the balance between ingestion and elimination. A simplified equation of uptake, retention and loss of heavy metals in top predators may be described:

UPTAKE = LOSS + RETENTION

There are three main uptake routes in marine mammals: food, transplacental transfer and in milk during suckling (Law *et al*, 1992). Diet is therefore a good measure of total body burden (Dehn *et al,* 2006). Metal uptake via ingested seawater or the lungs however, cannot be excluded in marine mammals (Law, 1996). The main excretion routes in seals are faeces, urine, hair, transplacental transfer and milk (Law *et al*, 1992). Contaminant loss can occur through metabolism but metals are conservative contaminants. They may be stored in a different form, such as demethylation of Hg, but they will still remain in the body tissues.

The simplified equation of uptake, retention and loss of heavy metals in adult seals may be described as:

INTAKE VIA FOOD = EXCRETION VIA FAECES + EXCRETION VIA URINE + RETENTION

Metal concentrations are also excreted via the hair of seals during the moult. Excretion via the hair must therefore be added into the equation. The simplified equation of uptake, retention and loss of heavy metals in adult seals may be extended:

UPTAKE VIA FOOD = (EXCRETION VIA FAECES + EXCRETION VIA URINE + EXCRETION VIA HAIR) + RETENTION

In breeding females there may also be loss via transplacental transfer and milk production.

6.2.1. Metal intake by the diet

In marine mammals, trace elements are mainly incorporated into the body via their food, and so diet is the main factor determining the trace element load of the given species (Bustamante *et al,* 2004). Levels of heavy metals in seals would be expected to vary with food quantity and

quality, particularly reflecting seasonal variation in food availability. Drescher *et al* (1977) assessed comparable studies of pollutant loads in seal tissue with pollutant loads in prey on the German North Sea coast. There was a tendency for higher concentrations of Cu, Zn and Cd in seal tissue than in plaice and cod tissue but there was no clear evidence of higher Pb concentrations in seals than in fish. Diet has been cited as playing an important role in determining the Cu, Cd and As burden of seals (McClurg, 1984; Kubota *et al*, 2001; O'Hara and O'Shea, 2001). High levels of Cu in the Ross seal, *Ommatophoca rossi*, are thought to be natural and to reflect the relatively high levels of Cu in squid, their main prey species (McClurg, 1984). High Cd levels in some marine mammals may be the result of feeding on prey species with naturally high Cd levels, especially squid, rather than external pollution (O'Hara and O'Shea, 2001). High concentrations of Cd are observed in marine mammals which consume a large proportion of cephalopods and crustaceans, such as Ross seals, whereas predominately fish eating species, for example harbour seals and grey seals, tend to have low Cd levels (Roberts *et al*, 1976; Drescher *et al*, 1977; Caines, 1978; Duinker *et al*, 1979; Bustamante *et al*, 2004). Cadmium concentrations are high in cephalopods and crustacean eating seals even where they inhabit areas remote from pollution sources, suggesting that food is the main Cd source. Arsenic concentrations in the livers of 226 individuals representing 16 different marine mammal species varied widely (Kubota *et al*, 2001). This variation was largely attributed to trophic level. Species feeding on cephalopods and crustaceans had higher As concentrations than those feeding on a mixed diet of cephalopods, crustaceans and fish and those feeding specifically on fish. Actual concentrations ranged from <0.10 to 7.68 mg kg^{-1} dry mass. These levels are lower than those in their prey and support laboratory studies that As does not biomagnify. Organoarsenic compounds, the predominant forms of As in marine organisms, have the potential to biomagnify but they are rapidly excreted through the urine.

Thompson (1990) reported that Hg was the only metal that appeared to biomagnify. Concentrations of Zn, Cu, Pb and Cd in the muscle and liver of prey fish did not differ significantly from the organs of marine mammals but Hg was considerably higher in the liver of seals than in the fish. Most of the Hg in fish and terrestrial mammals is in the form of methylHg. There have been a number of experiments conducted where seals have been fed methylHg in their diets (Law, 1995). Two harp seals fed high methylHg doses of 25 mg kg^{-1} of

body mass per day died within twenty and twenty-six days. The cause of death was ascribed to chronic renal failure. The concentrations of methylHg in the livers at death were 127 and 125 mg kg^{-1}, with total Hg concentrations of 134 and 142 mg kg^{-1}; that is most of the ingested Hg was still in the methyl form. Two harp seals fed lower doses of 0.25 mg kg^{-1} of body mass for 60 and 90 days exhibited lower concentrations of 18 mg kg^{-1} methylHg and 64 mg kg^{-1} total Hg after 60 days, and 76 mg kg^{-1} methylHg and 83 mg kg^{-1} total Hg after 90 days. Only in an area such as the Mediterranean Sea where Hg concentrations in fish are particularly high (up to 7 mg kg^{-1} in muscle of some species) could the dietary intake of wild marine mammals approach even the lower doses quoted in these dietary experiments but they do indicate that food is a main source of heavy metal intake.

To quantify the metal intake by predators it is necessary to determine the quantity, species and size of prey consumed. Norday and Blix (1988) reported that adult harbour seals would consume approximately 2.5 to 5% of their body mass per day. The quantity of food consumed is dependent on the calorific value. Seals require a lower mass of a prey species with a higher energetic density compared with prey species with a lower value (Prime and Hammond, 1987). The energetic demands for maintenance of the harbour seal is approximately 68 kcal body mass kg^{-1} day and 95 kcal/body mass kg^{-1} day, for adults and sub-adults respectively (Härkönen and Heide-Jørgenson, 1991). Additionally, a 70 kg adult harbour seal will expend 39 calories per metre travelled. The energetic demands of a seal are therefore dependent on the distance traveled, which is related to the abundance and location of the prey species.

Food requirements also depend on the size of a population and the sex and age structure of that population (Tollit, 1996). Energy demand can vary seasonally, such as decreased food intake during the breeding season and an increase in food intake after moult. Net energy gain is calculated from gross energy gain by taking account of digestive efficiency, the energy loss in faeces, urine and heat increment associated with feeding (Ronald *et al*, 1984). The quantity and calorific quality of prey consumed and the metal intake is affected by seasonal, annual and geographical differences in the diet (Olesiuk, 1993; Boyd *et al*, 1994). A multi-

species model is therefore required to determine seasonal prey selection including predator response to prey changes, such as switching to alternative food resources.

6.2.2. Metal intake by seal pups and output by adult, female seals via transplacental transfer and milk

Dehn *et al* (2005) reported that Zn and Cu readily cross the placental barrier and MT is high in fetuses, probably due to increased demand for these essential metals by developing and growing tissues. Law *et al* (1992) also reported higher Cu concentrations in young animals and neonates compared to adults, probably due to considerable transplacental transfer. Cu levels remained high during suckling and then gradually decreased in the adult. Law *et al* (1992) however, reported that Zn was taken in via milk and food rather than from the placenta. They found little transplacental transfer of Zn in marine mammals with concentrations in one neonatal harbour porpoise liver and one foetal common dolphin liver being only half and one third, respectively of levels detected in their mothers.

Lead is readily transferred across the placenta in humans, rats and goats and this also seems to be the case in marine mammals (André *et al*, 1991). The concentrations of Pb in the livers of a neonatal harbour porpoise and a foetal common dolphin were approximately 35% of those detected in their mothers (Law *et al*, 1992). Lead was also transferred from mother to calf via milk in the striped dolphin (André *et al*, 1991). Movement of Pb across the placenta however, was not significant in the harbour seals studied by Roberts *et al* (1976). Lead was detected in milk at low quantities. Cadmium levels were very low in the livers of neonatal harbour porpoise and the foetus of a common dolphin, suggesting negligible transplacental transfer (Law *et al*, 1992). Renal and hepatic Cd concentrations were significantly higher in female grey seals than males from the Faroe Islands suggesting that, together with higher ratios of food ingested to body mass, that the transfer of Cd to the foetus through the placenta or to calves via milk is not an important excretion route for females (Bustamante *et al*, 2004). In contrast, Teigen *et al* (1999) measured high levels of Cd and Hg in fetal hepatic and renal tissues of grey seals, indicating significant placental transport. Freeman and Horne (1973) reported however, that concentrations of Hg in fetal and neonatal marine mammals were normally low suggesting low placental transfer of Hg to developing pups. This was supported by liver concentrations of methylHg in grey seal pups from the Farne Islands being only one-tenth of those found in the

mother (van de Ven *et al*, 1979). Arsenic concentrations measured in the liver and kidney tissues of two fetuses were lower than those measured in adults, suggesting that the degree of transplacental transfer is low (Philips, 1990).

6.2.3. Metal output by seals via hair

Trace metal accumulation in hair samples of the harbour seal were analyzed by Wenzel *et al* (1993). Female seals had lower Cd concentrations in hair than male seals. Cadmium and Pb were observed to accumulate with age in hair samples. Hg content in hair was several times higher than in skin. Mercury concentrations in the hair were significantly higher than Pb, which significantly exceeded Cd levels. A connection between metal accumulation and moult was recognised. The moult season for harbour seals in the UK is between late July and August (Anderson, 1990).

6.2.4. Metal output via faeces and urine

Metals recovered from faeces consist of material that has been ingested, but not absorbed across the gut wall, and metals excreted from the body, mainly in the bile. The proportion of a particular metal recovered in the faeces depends on the specific rates of absorption and excretion. In mammals only about 5-6% of ingested Cd and <10% of ingested Pb is absorbed across the gut wall (Mason and MacDonald, 1986) and most faecal Cd and Pb represents unabsorbed material. Some 95% of ingested methyl Hg is absorbed across the gut wall (Kazantzis, 1980) so faecal Hg content will be a poor estimator of dietary intake.

Mason and MacDonald (1986) analysed over 500 otter, *Lutra lutra* faecal samples for Cd, Hg and Pb. Analysis of faeces was used as a technique to indirectly assess pollutant exposure in otter populations. The mean concentrations of metals ranged from 1.53 to 3.97 mg kg^{-1} dry mass of Cd, 0.25 to 0.74 mg kg^{-1} dry mass of Hg and 12.4 to 20.9 mg kg^{-1} dry mass of Pb. Faecal samples from common tern chicks were used to determine metal loads (Quirke, 1995). Mean metal concentrations were 186.6 mg kg^{-1} of Zn, 55.2 mg kg^{-1} of Pb, 14.4 mg kg^{-1} of Cu and 3.9 mg kg^{-1} of Cd. In birds urine and faeces are voided together and hence it is likely that both were collected and analysed. There was no significant temporal variation in this study conducted between March and June.

The urine of seals is thought to be mostly, if not all, from ingested water from the food (Ronald *et al*, 1984), together with nitrogenous and other waste material unfiltered from the kidneys. The urine could potentially be an important excretory route for metals.

6.3. METHODOLOGY

6.3.1. Collection of body tissues from seals

Two dead seals were recovered from Seal Sands for analysis of heavy metals. A 4 year old, male grey seal was recovered from Seal Sands in August, 2000 and a 2 year old, female harbour seal was recovered from Greatham Creek in January, 2003. A post mortem was carried out on these two seals by a veterinarian. Sections of liver, kidney, blubber, brain, heart, lung and flipper were taken from the former seal and all but the heart was taken for the latter. Samples of other body tissues, especially muscle and bone were not taken by the veterinarian. The body tissues were frozen in labeled plastic pots.

6.3.2. Collection of seal faecal samples

Seal faecal samples were collected from Greatham Creek, a tributary of the Tees Estuary. This ensured that the samples were almost certainly from harbour seals, since grey seals were not observed to haul out at Greatham Creek. In addition, it is the most accessible and safely traversed site of the Tees seals haul out sites and it is further above sea level than the other haul out sites. It is not immersed during spring tides so faeces can collect over a number of days whereas the other haul-out sites were immersed daily by the tide. Collections were therefore most successful at the end of the spring tides and fortnightly collections were made. Foot and Mouth Disease restrictions prevented seal faecal samples being collected during the summer of 2001. A flat bladed knife was used to lift the faecal samples into individual plastic pots. They were labeled and stored at -20^0C until further processing.

6.3.3. Sample preparation and digestion of seal body tissues and seal faecal samples

The tissue samples of the two adult seals were defrosted and cut up using a scalpel. The samples were dried to constant weight in the oven (at 60°C in order to avoid fat evaporation). Samples were digested and analysed for Zn, Cu, Pb, Cd, As and Cr using the method described in Sections 4.2.2 and 4.2.3. Samples were digested and analysed for Hg using the method described in Sections 4.2.5.

6.3.4. Replication of samples

Five samples of each seal body tissue were analysed and the average concentration was determined to prevent bias from non-homogenised material. Some individual faecal samples were very large and only part of the sample was used for analysis. The sample was homogenised but to achieve a good estimate of the total metal concentration replicates were conducted and the average given. Analysis was conducted on seal faecal samples collected bimonthly (Table 6.1).

Table 6.1. Bi-monthly sample size of seal faecal samples collected from the Tees Estuary, 2000-2002

Monthly periods	No. of samples
January-February	10
March - April	16
May-June	14
July-August	18
September-October	21
November-December	9

6.3.5. Calculation of tissue burden in seals

The percentage of body composition represented by each organ was not measured for the two seals recovered from Seal Sands because the veterinarian did not record this information and only sections of the organs were given to the author. The percentage of body composition for harbour seals and grey seals was not available in the literature. Percentage body composition was therefore estimated from data collected from male and female Ross seals and crabeater seals, *Lobodon carcinophagus* (Bryden and Erickson, 1976) and three adult Weddell seals, *Leptonychotes weddelli* (Bryden *et al,* 1984) (Table 6.2). The body composition of the different seal species may vary and hence, this may have lead to some bias. Metal concentrations were only analysed in the organs and blubber of the harbour seal and the grey seal. The remainder of the body of crabeater and Ross seals was composed mainly of muscle (44 %), bone (10 %), skin (8 %) and blood (14 to 15 %) (Bryden and Erickson, 1976; Bryden *et al,* 1984). It was assumed that metal concentrations in these body tissues would be similar to those in the heart (which is a vascularized muscle) to allow the total body burden of metals in each seal to be estimated.

Table 6.2. Percentage of body composition represented by each organ for the two seals recovered from Seal Sands, 2000 and 2003.

Seal	Organ	% body composition
Male, grey seal	**Liver**	1.7 – 2.9 [2]
	Kidney	0.1 – 0.3 [2]
	Blubber	23.9-33.5 [2]
	Brain	0.2 – 0.5 [1]
	Heart	0.5 - 0.6 [2]
	Lungs	1.0 – 1.6[2]
Female, harbour Seal	**Liver**	1.6 – 3.1 [2]
	Kidney	0.2 – 0.4 [2]
	Blubber	23.9–33.5 [2]
	Brain	0.2 – 0.5 [1]

[1] Bryden and Erickson, 1976; [2] Bryden *et al,* 1984

Mass per organ (kg) = (% of body composition per organ /100) x total body mass (kg)

The tissue burden of metals was estimated from metal concentrations and the mass of each body organ:

Metal concentration per body organ (mg kg^{-1}) = metal concentration per mg kg^{-1} x body mass of organ

6.3.6. Statistical analysis of seal faecal samples

The Kolmogorov-Smirnov statistic was used to test the goodness of fit for normal distribution and log-normal distribution for metal concentrations in seal faecal samples (Table 6.3). Not all of the data was normally distributed even after the data was logged so non-parametric tests were used for consistency between tests on each metal.

Table 6.3. Test of the goodness of fit of data and log transformed data to a normal distribution (Kolmogorov-Smirnov test)

	Zn	Cu	Pb	Cd	As	Cr
Actual	**	*	**	***	**	***
Log	NS	NS	NS	***	NS	NS

Concentrations of essential metals excreted in faecal samples are expected to vary less than non-essential metals due to regulation at metabolically required levels (Law, 1995; Bustamante *et al,* 2004). Coefficient of variation of log transformed metal concentrations was used to compare the extent of variability in levels of metals in seal faecal samples. The calculation was:

Coefficient of variance (CV) = standard deviation / mean.

Spearman rank correlation was used to analyse the interaction between pairs of metal concentrations within faecal samples. This is a non-parametric test and data for metal concentrations were not normally distributed.

There were too few faecal samples to test the statistical difference between bi-monthly metal concentrations. The statistical difference between seal faecal samples collected in the winter (September to February) and the summer (March to August) were compared using the Mann-Whitney U test.

6.3.7. Metal budgets in seals

The mass of each prey species consumed by seals was estimated in Chapter 2 from the faecal samples. The mass of prey species consumed bi-monthly can be calculated from the proportions of each prey species consumed bi-monthly shown in Chapter 3. The mass of prey species consumed bi-monthly was then multiplied by the bi-monthly median metal concentrations for that prey species given in Chapter 4. These metal concentrations were converted into wet mass so that they could be compared with metal concentrations in the wet mass of seals. The mass of Crustacea consumed could not be calculated from the prey remains in faeces so median metal concentrations in whole Crustacea were used.

The metal burden consumed daily by each individual was estimated. The wet mass of seal faecal samples containing prey remains collected during each bimonthly period was multiplied by the metal concentrations measured in the wet mass of seal faeces. This was then divided by number of seal faeces collected bi-monthly that had prey present to estimate

the metal burden taken in from the diet from each individual seal faecal sample (Table 6.4). The metal burden in one seal faecal sample was assumed to represent the daily metal burden consumed by an individual seal.

Table 6.4. The bi-monthly number of seal faecal samples with prey present.

Month	No. of faeces with prey present
Jan-Feb	22
Mar-Apr	16
May-June	30
Jul - Aug	32
Sept- Oct	19
Nov- Dec	19

The median output of metals in the seal faeces (mg) was calculated by multiplying the metal concentrations (mg kg^{-1} wet mass) by the total wet mass (kg) of each faecal sample. The median metal concentrations and inter-quartile range for each bi-monthly period was compared with the estimated metal budget taken in by the seals from their prey in order to estimate the daily retention of metals by seals.

Metal concentrations were not analysed in Chapter 4 for poor cod (116 consumed by seals) and haddock (1 consumed by seals). It was assumed that metal concentrations in poor cod and haddock species would be similar to those in cod, based on their similar lifestyle and size, and therefore metal concentrations in cod were substituted for these species.

6.4. RESULTS

6.4.1. Metal concentrations in seal body tissues from the Tees Estuary

Heavy metal concentrations were measured in a two year old, female harbour seal and a four year old male, grey seal. This is a very small sample size and the results are only a subjective indication of metal concentrations in two seals from the Tees Estuary. Metal concentrations were analysed in dry mass of seal body tissues to be comparable with the metal concentrations in their prey species shown in Chapter 4 and 5 (Table 6.5).

Table 6.5. Comparison of metal concentrations (mg kg^{-1} dry mass) between two seals recovered from Seal Sands, 2000 (grey seal) and 2003 (harbour seal) (ND = Not Detected, NM = Not measured)

Seal	Organ	Zn	Cu	Pb	Cd	As	Cr	Hg
Grey Seal	**Liver**	87.6	31.5	0.6	0.1	4.2	0.3	500.0
	Kidney	49.2	2.5	0.1	0.3	1.1	0.4	4.0
	Blubber	2.4	0.2	0.02	ND	5.2	0.7	38.0
	Brain	48.7	9.3	ND	0.03	2.8	1.2	ND
	Heart	56.9	2.9	0.6	0.02	2.4	0.3	2.0
	Lungs	41.1	0.9	ND	0.03	1.8	0.3	ND
Harbour Seal	**Liver**	93.2	16.5	0.3	0.1	4.8	0.3	280.0
	Kidney	62.0	4.4	0.5	0.2	3.2	0.5	35.0
	Blubber	2.7	0.6	ND	0.05	4.6	1.2	NM
	Brain	44.6	6.6	0.3	0.04	4.8	1.7	100.0

Metal concentrations were analysed in wet mass of seal body tissues to be comparable with most literature sources and also to allow total metal burdens in organs to be estimated (Table 6.6).

Table 6.6. Comparison of metal concentrations (mg kg^{-1} wet mass) between two seals recovered from Seal Sands, 2000 (grey seal) and 2003 (harbour seal) (ND = Not Detected, NM = Not measured).

Seal	Organ	Zn	Cu	Pb	Cd	As	Cr	Hg
Grey Seal	**Liver**	12.1	4.5	0.1	0.01	0.6	0.04	71.4
	Kidney	4.8	0.2	0.03	0.02	0.1	0.04	0.6
	Blubber	0.3	0.03	ND	ND	0.9	0.1	5.4
	Brain	6.7	1.3	ND	ND	0.4	0.2	ND
	Heart	6.1	0.3	0.06	ND	0.3	0.04	0.3
	Lungs	5.2	0.2	ND	ND	0.3	0.05	ND
Harbour Seal	**Liver**	13.3	2.5	0.05	0.02	0.7	0.0	36.8
	Kidney	7.1	0.5	0.06	0.02	0.4	0.06	4.9
	Blubber	0.4	0.1	ND	ND	0.6	0.2	NM
	Brain	6.2	0.9	0.04	ND	0.07	0.2	13.8

Metal concentrations were estimated in relation to the percentage of body mass represented by each body organ (Table 6.7).

Table 6.7. Total metal burden in the body organs (mg) of two seals recovered from Seal Sands, 2000 (grey seal) and 2003 (harbour seal) (ND = Not Detected, NM = Not measured)

Seal	Organ	Estimated mass of organ (kg)[1]	Zn	Cu	Pb	Cd	As	Cr	Hg
Male grey seal (178 kg)	**Liver**	3.0 - 5.5	36.3– 66.6	13.5-24.8	0.3 – 0.6	0.03-0.06	1.8-2.7	0.1-0.2	214.2-392.7
	Kidney	0.4 - 0.5	1.9 -2.4	0.01	0.01–0.02	0.01	0.04-0.05	0.02	0.2-0.3
	Blubber	42.5– 60.0	12.8 -18.0	1.3 – 1.8	ND	ND	38.3-54.0	4.3 - 6.0	229.5-324.0
	Brain	0.4 – 0.9	2.7 – 6.0	0.5 – 1.2	ND	ND	0.2-0.4	0.1-1.1	ND
	Heart	0.9 – 1.1	5.5 – 6.7	0.3	0.05 – 0.06	ND	0.3	0.04	0.3
	Lungs	1.8 – 2.8	9.4 –14.6	0.4 – 0.6	ND	ND	0.5-0.8	0.1	ND
Female harbour seal (48 kg)	**Liver**	0.7 – 1.5	9.3 – 20.0	1.8 – 3.8	0.04 – 0.08	ND	0.5- 1.1	ND	25.8 - 55.2
	Kidney	0.1 – 0.2	0.7 – 1.4	0.05– 0.1	ND	ND	0.04 – 0.1	ND	0.5 – 1.0
	Blubber	11.5-16.0	4.6 – 6.4	1.2 – 1.6	ND	ND	6.9 - 9.6	2.3-3.2	NM
	Brain	0.1-0.2	0.6 -1.2	0.09– 0.2	ND	ND	ND	0.02-0.04	1.4 -2.8

[1] **based on % body composition of crabeater and Ross seals (Bryden and Erickson, 1976; Bryden *et al*, 1984).**

In both seals, Zn was highest in the liver, followed by the blubber, Cu was highest in the liver, Pb and Cd were low in all organs but highest in the liver and As and Cr were highest in the blubber. The Hg content in the liver of both seals and the blubber of the grey seal was considerably higher than other metals.

6.4.2. Metal concentrations in seal faeces from the Tees Estuary

Zinc, Cu, Pb, Cd, As, Cr and Hg concentrations in seal faeces were presented as medians and inter-quartile range (Table 6.8).

Table 6.8. Median and inter-quartile range for metal concentrations (mg kg^{-1} dry mass) in seal faecal samples and number of samples (n)

	Median (mg kg^{-1} dry mass)	25% and 75% quartiles (mg kg^{-1} dry mass)	n
Zn	56.6	25.3 - 140.0	89
Cu	12.1	7.5 - 17.3	89
Pb	14.5	6.8 - 34.5	89
Cd	0.2	0.1 - 0.8	89
As	2.3	0.6 - 5.2	89
Cr	8.4	5.1 - 14.7	89
Hg	15.0	7.0 - 30.0	39

Coefficients of variance (CV) were used to compare the extent of the variation between log transformed metal concentrations in seal faecal samples (Figure 6.1). Concentrations of essential metals excreted in faecal samples are expected to vary less than non-essential metals.

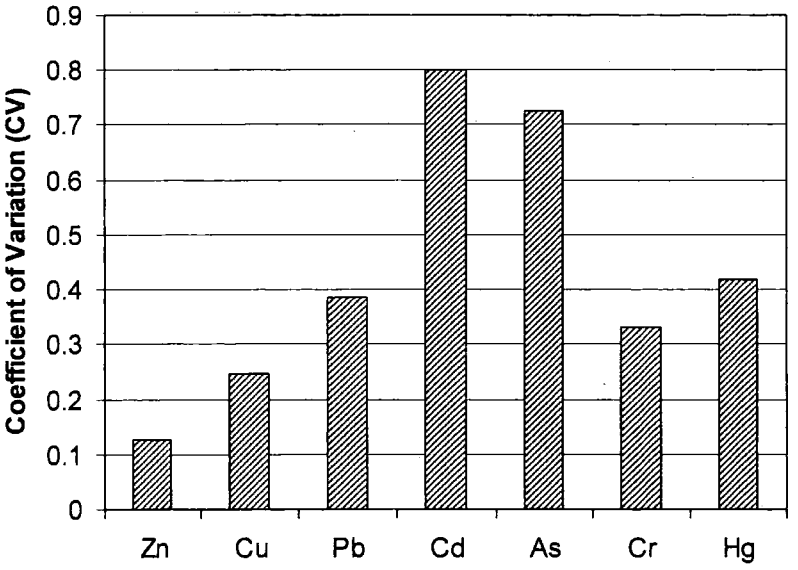


Figure 6.1. Coefficients of variation for Zn, Cu, Pb, Cd, As, Cr and Hg in seal faeces

The highest to lowest ranks of coefficients of variation were:

Cd > As > Hg > Pb > Cr > Cu > Zn

6.4.2.1. Interaction between metal elements in seal faeces

Correlations were conducted between the seven metal concentrations for each of the faecal samples to determine whether the metal concentrations correlate with other metal and hence, may have similar routes of uptake and sources of exposure (Table 6.9). In the case of one metal having a high concentration for any given individual faecal sample, then all metals might be expected to have high concentrations in that individual sample. Conversely, in the case of one metal having a low concentration in an individual faecal sample then all metals might be expected to have low concentrations in that individual sample.

Table 6.9. Spearmans correlation between different metals in faecal samples (Notes: NS = No significant correlation between metals, * = Significant correlation (p<0.05), ** = Significant correlation (p<0.01), * = Significant correlation (p<0.001))**

	Cu	Pb	Cd	As	Cr	Hg
Zn	NS	NS	-0.650***	-0.320*	0.330**	NS
	Cu	0.688***	NS	0.520***	0.700***	0.441*
		Pb	NS	0.600***	0.741 ***	0.406*
			Cd	NS	0.298 **	NS
				As	0.689 ***	NS
					Cr	NS
						Hg

6.4.2.2. Seasonal variation between metal concentrations in seal faecal samples

There was seasonal variation in faecal metal concentrations between winter (September to February) and the summer (March to August) for Zn ($p< 0.001$), Cd ($p< 0.001$) and Cr ($p < 0.05$) (Mann-Whitney U test). Metal concentrations in seal faeces were higher in winter than in summer, except for Zn. Zn concentrations were significantly higher in summer than winter months.

6.4.3. Metal budgets in seals

The bi-monthly metal burden in seals from the Tees Estuary was estimated from the uptake and loss of metal concentrations. The bi-monthly uptake of metal concentrations by each seal was estimated from the biomass of each prey species present in faecal samples collected for each bi-monthly period and the median metal concentrations for each prey species. The total biomass of each fish species consumed in each bi-monthly period by all seals was estimated in Chapter 3. This biomass of each fish species for each bi-monthly period was multiplied by the bi-monthly median metal concentrations for that species, calculated in Chapter 4. Metal concentrations were not measured in poor cod so the bi-monthly median metal concentration in cod was used as it was assumed that concentrations would be similar between these two species. The mass of Crustacea species could not be estimated from the prey remains in faeces as they were fragmented. A total biomass was estimated from the median mass of common shrimp and shore crab collected in the power station intake water for each bi-monthly period. This total biomass was multiplied by the median metal concentrations in whole Crustacea, although this metal concentration may be an over-estimate if the concentrations in the exoskeleton are not bioavailable as discussed in Chapter 5. The biomass and metal concentrations for each prey species were tabulated into a spreadsheet to calculate the metal burden for all seals in one given bi-monthly period. An example of this spreadsheet is given to calculate zinc burdens in seals during January to February (Table 6.10). The total metal burden is calculated for all faecal samples collected in any bi-monthly period. This total metal burden was divided by the number of faecal samples (23 faecal samples for the period January-February (Table 6.10)) analysed in each bi-monthly period to provide an estimate of daily metal burden for each individual seal.

Table 6.10. Example calculation of metal burden in seals for the period of January to February

Prey species	Biomass (kg)	Median metal concentration (mg/kg)	Total metal burden for all seals (mg)	Total metal burden per seal (mg)
Herring	0.2	90.5 (10.5-280.5)	18.1 (2.1-56.1)	0.8 (0.1-2.4)
Sprat	0.01	50.0 (20.0 -140.0)	0.5 (0.2- 1.4)	0.02 (0.01-0.06)
Cod	6.9	53.6 (52.1 -93.9)	370.3 (359.5-647.9)	16.1 (15.6 -28.2)
Whiting	0.6	64.5 (34.6-195.0)	38.5 (19.8 -114.0)	1.7 (0.9- 5.0)
Saithe	0.3	50.4 (27.6-56.6)	14.2 (8.3 -17.0)	0.6 (0.4 -0.7)
Poor cod	4.6	54.0 (52.4-94.5)	247.4 (238.0-433.8)	10.8 (10.6 - 18.8)
Flounder	0.2	105.2 (47.8 -237.8)	20.8 (9.4 - 45.6)	0.9 (0.4 - 2.1)
Plaice	0.06	97.7 (54.5-128.2)	5.9 (3.3 -7.7)	0.3 (0.1 -0.3)
0-1 year pleuronectids	0.2	114.4 (51.9 -258.4)	22.6 (10.3 – 50.3)	0.9 (0.5 – 2.2)
Shore crab	0.2	72.5 (61.0 -139.0)	14.5 (12.2 -27.8)	0.7 (0.5 – 1.2)
Total			**754.4 (669.5-1415.4)**	**32.8 (29.2 – 61.0)**

The estimate of daily metal burden for each individual seal is shown in Table 6.11. This assumes that seals only defecate once daily. The estimated daily metal burden per individual seal was highest during the winter months and considerably lower in July to August.

Table 6.11. Estimated median daily metal burden (mg) per individual seal faeces for each bi-monthly period (minimum and maximum burdens in brackets).

	Zinc (mg)	Copper (mg)	Lead (mg)	Cadmium (mg)	Arsenic (mg)	Chromium (mg)
Jan-Feb	32.8 (29.2-61.0)	1.6 (1.1-2.6)	1.9 (0.8-3.0)	0.2 (0.1-0.3)	5.1 (2.0-9.7)	0.5 (0.5-0.6)
Mar-Apr	15.9 (9.3-39.4)	0.5 (0.2-0.9)	0.5 (0.2-1.4)	0.1 (0.02-0.2)	2.9 (1.1-6.3)	0.2 (0.1-0.9)
May-Jun	27.8 (26.0-30.8)	0.8 (0.6-1.2)	0.7 (0.5-1.1)	0.1 (0.07-0.1)	3.1 (2.8-4.3)	0.5 (0.4-0.6)
Jul-Aug	4.3 (2.5-7.3)	0.2 (0.1-1.2)	0.2 (0.1-0.7)	0.02 (0.01-0.08)	0.6 (0.3-2.1)	0.07 (0.04-0.2)
Sept-Oct	18.1 (7.5-40.9)	0.5 (0.2-1.5)	0.6 (0.2-1.8)	0.05 (0.02-0.2)	1.5 (0.2-6.3)	0.2 (0.1-0.5)
Nov-Dec	18.3 (9.7-34.9)	0.5 (0.2-4.5)	0.8 (0.3-2.6)	0.05 (0.02-0.2)	1.8 (0.4-8.8)	0.3 (0.1-0.7)

The amount of metal retained seasonally was calculated from the estimated daily metal burden taken in by seals from their diet minus the measured mean output of metals in the faeces (Table 6.12).

Table 6.12. Estimate of median daily metal burden taken in by seals from the diet (mg), median output of metals in seal faeces (mg) and the estimated range of metal retention per seal (mg)

a) zinc	Estimated median daily metal burden taken in from the diet per seal (mg)	Median and interquartile range of output of metals in seal faeces (mg)	Estimated range of metal retention per seal (mg)
Jan-Feb	32.8	2.5 (0.2-4.0)	28.8 – 32.6
Mar-Apr	15.9	0.1 (0.08-0.2)	15.7 – 15.8
May-Jun	27.8	0.2 (0.1-0.3)	27.5 – 27.7
Jul-Aug	4.3	2.3 (1.6-3.3)	1.0 – 2.7
Sept-Oct	18.1	2.1 (1.1-4.2)	13.9 – 17.0
Nov-Dec	18.3	1.6 (0.4-4.4)	13.9 – 17.9
b) copper			
Jan-Feb	1.6	0.2 (0.1-0.5)	1.1 – 1.5
Mar-Apr	0.5	0.05 (0.03-0.08)	0.4 - 0.5
May-Jun	0.8	0.1 (0.08-0.2)	0.6 – 0.7
Jul-Aug	0.2	0.2 (0.1-0.3)	-0.1 – 0.1
Sept-Oct	0.5	0.2 (0.1-0.3)	0.2 – 0.4
Nov-Dec	0.5	0.2 (0.08-0.4)	0.1 – 0.4
c) lead			
Jan-Feb	1.9	0.3 (0.1-0.8)	1.1 – 1.8
Mar-Apr	0.5	0.07 (0.03-0.1)	0.4 – 0.5
May-Jun	0.7	0.2 (0.08-0.4)	0.3 – 0.6
Jul-Aug	0.2	0.3 (0.1-0.6)	-0.4 – 0.1
Sept-Oct	0.6	0.2 (0.1-0.4)	0.2 – 0.5
Nov-Dec	0.8	0.3 (0.1-0.3)	0.5 – 0.7
d) cadmium			
Jan-Feb	0.2	0.003 (0.002-0.004)	0.2
Mar-Apr	0.1	0.005 (0.005-0.009)	0.1
May-Jun	0.1	0.007 (0.003-0.01)	0.1
Jul-Aug	0.02	0.003 (0.002-0.003)	0.02
Sept-Oct	0.05	0.003 (0.002-0.005)	0.05
Nov-Dec	0.05	0.009 (0.002-0.02)	0.05
e) arsenic			
Jan-Feb	5.1	0.07 (0.01-0.1)	5.0 – 5.1
Mar-Apr	2.9	0.007 (0.004-0.02)	2.9
May-Jun	3.1	0.06 (0.02-0.1)	3.0 – 3.1
Jul-Aug	0.6	0.04 (0.007-0.07)	0.5 - 0.6
Sept-Oct	1.5	0.02 (0.01-0.06)	1.4 – 1.5
Nov-Dec	1.8	0.03 (0.006-0.07)	1.7 - 1.8
f) chromium			
Jan-Feb	0.5	0.2 (0.1-0.3)	0.2 – 0.4
Mar-Apr	0.2	0.04 (0.03-0.08)	0.1 – 0.2
May-Jun	0.5	0.1 (0.08-0.3)	0.2 – 0.4
Jul-Aug	0.07	0.2 (0.05-0.3)	-0.2 – 0.02
Sept-Oct	0.2	0.1 (0.06-0.2)	0.0 – 0.1
Nov-Dec	0.3	0.1 (0.09-0.1)	0.2

The estimated retention of metals varied considerably between seasons with the lowest retention in July to August for all metals. Metal retention was highest in January to February for all metals, although metal retention in Cr was the same in March to April as January to February.

The total metal burden in the two seals recovered from Seal Sands was estimated. The two seals were different species, sex and age. The grey seal was four years old and the harbour seal was two years old. These differences may have affected the results so the two seals are not directly comparable. Metal concentrations were only measured in 28 to 40 % of the total grey seal body mass and 26 to 37 % of the total harbour seal body. The remainder of the body composition is mainly muscle, bone, skin and blood. It was assumed that metal concentrations in these body tissues would be similar to that in the heart (which is a vascularized muscle) and the total body burden for each seal was estimated. Whilst metal concentrations in the heart and the muscle are likely to be similar there is also an assumption that metal concentrations will be similar in the heart and the bone and this is unlikely for some metals, particularly lead. Lead concentrations tend to be high in bone so the estimated total lead burden is likely to be an under-estimate. The total mercury burden was not included because mercury was only analysed in two prey species.

The estimated total metal burden in the two seals was compared to the range of estimated metal retained in seals (Table 6.13).

Table 6.13. Estimated total metal burden (mg) in two seals recovered from Seal Sands compared with the range of all estimated metal retention values (mg)

Seal	Organ	Zn	Cu	Pb	Cd	As	Cr	Hg
Male grey seal (178 kg)	**Liver**	36.3– 66.6	13.5-24.8	0.3 – 0.6	0.03-0.06	1.8-2.7	0.1-0.2	214.2-392.7
	Kidney	1.9 -2.4	0.01	0.01–0.02	0.01	0.04-0.05	0.02	0.2-0.3
	Blubber	12.8 -18.0	1.3 – 1.8	ND	ND	38.3-54.0	4.3 - 6.0	229.5-324.0
	Brain	2.7 – 6.0	0.5 – 1.2	ND	ND	0.2-0.4	0.1-1.1	ND
	Heart	5.5 – 6.7	0.3	0.05 – 0.06	ND	0.3	0.04	0.3
	Lungs	9.4 –14.6	0.4 – 0.6	ND	ND	0.5-0.8	0.1	ND
	Other (estimate)	653.9-786.9	32.2-38.7	6.4-7.7	0.2-0.3	32.2-38.7	4.3-5.2	32.2-38.7
	Total	**722.5-901.2**	**48.2-67.4**	**6.8-8.4**	**0.2-0.4**	**73.3-97.0**	**9.0-12.7**	**476.4-756.0**
Female harbour seal (48 kg)	**Liver**	9.3 – 20.0	1.8 – 3.8	0.04 – 0.08	ND	0.5- 1.1	ND	25.8 - 55.2
	Kidney	0.7 – 1.4	0.05– 0.1	ND	ND	0.04 – 0.1	ND	0.5 – 1.0
	Blubber	4.6 – 6.4	1.2 – 1.6	ND	ND	6.9 - 9.6	2.3-3.2	NM
	Brain	0.6 -1.2	0.09– 0.2	ND	ND	ND	0.02-0.04	1.4 -2.8
	Other (estimate)	**183.6-217.2**	**9.0-10.7**	**1.8-2.1**	**0.006-0.007**	**9.0-10.7**	**1.2-1.4**	**9.0-10.7**
	Total	**198.8-246.2**	**12.1-16.4**	**1.8-2.2**	**0.006-0.007**	**16.4-69.0**	**3.5-4.6**	**36.7-69.7**
Estimated metal retention (mg) / seal daily		**1.0 – 32.6**	**-0.1 – 1.5**	**-0.4 – 1.8**	**0.02 – 0.2**	**0.5 – 5.1**	**-0.2 – 0.4**	**Not recorded**

The estimated total metal burden in the grey seal was considerably higher than in the harbour seal. The estimated total burden of Zn, Cu, As and Cr in the two seals was considerably higher than the estimated retention per seal daily. The estimated total burden of Pb was considerably higher in the grey seal than the estimated retention per seal daily, whereas in the harbour seal it was only slightly higher. The estimated total burden of Cd was slightly higher in the grey seal than the estimated retention per seal daily, whereas the total burden of Cd in the harbour seal was lower than the estimated retention per seal daily. The estimated metal retention of Hg in seals per day could not be calculated since the metal retention is based on the input from prey and output from faeces and Hg concentrations were not measured in all prey items.

6.5. DISCUSSION OF HEAVY METALS IN HARBOUR SEALS

6.5.1. Metal concentrations in body tissues of Tees Estuary seals

Zinc, Cu, Pb, Cd, As and Hg concentrations in the body tissues of the two seals found on the Tees Estuary were lower than concentrations reported in literature (Koeman *et al,* 1973; Reijnders, 1980; Thompson, 1990; Law *et al,* 1991; 1992; Simmonds *et al,* 1993) (Tables 6.14 to 6.18). Metal concentrations measured during the 1970s are expected to be lower than more recent measurements since metals discharge into the estuaries was not as well controlled. Metal concentrations measured during the 1970s were higher in harbour seals from the East Coast of England compared with other European locations.

Table 6.14. Zinc concentrations (mg kg^{-1} wet mass) in harbour seals from data in Thompson (1990) and present data from the Tees Estuary (2003).

Location	Age	Tissue	Concentration (mg kg^{-1} wet mass)	Date
Dutch Wadden sea	Adult	Liver	16-64	1979
		Kidney	15-25	
		Blubber	3-14	
		Brain	8-27	
		Spleen	26-31	
		Heart	31	
		Placenta	11	
Dutch Wadden sea	Fetus	Liver	89.0	
		Brain	8.0	
East Coast, England	Adult	Liver	43-84	1975
		Kidney	28-51	
		Blubber	4-13	
		Brain	19-36	
		Spleen	28-35	
		Heart	28-32	
		Placenta	33-35	
German North Sea	Adult	Liver	27-56	1975
		Kidney	16.3-32.5	
		Brain	10.8-15.0	
German coastal waters	Adult	Liver	27-60	1972
		Kidney	15.5-34	
		Muscle	15-36	
Tees Estuary	2 year old	Liver	13.3	2003
		Kidney	7.1	
		Blubber	0.4	
		Brain	6.2	

Table 6.15. Copper concentrations (mg kg^{-1} wet mass) in harbour seals from data in Thompson (1990) and present data from the Tees Estuary (2003).

Location	Age	Tissue	Concentration (mg kg^{-1} wet mass)	Date
Dutch Wadden sea	Adult	Liver	2.0-20.0	1979
		Kidney	4.8-5.1	
		Blubber	0.09-3.0	
		Brain	2.5-9.5	
		Spleen	3.3-4.0	
		Heart	5.8-8.2	
		Placenta	2.0	
Dutch Wadden sea	Fetus	Liver	49.0	
		Brain	<1.0	
German coastal waters	Adult	Liver	2.6-17	1972
		Kidney	2.3-4.0	
		Muscle	0.8-2.5	
Tees Estuary	2 year old	Liver	2.5	2003
		Kidney	0.5	
		Blubber	0.1	
		Brain	0.9	

Table 6.16. Lead concentrations (mg kg^{-1} wet mass) in adult harbour seals from data in Thompson (1990) and present data from the Tees Estuary (2000 and 2003).

Species	Location	Tissue	Concentration (mg kg^{-1} wet mass)	Date
Grey seals	East Coast, Scotland	Liver	Up to 17.0	1975
Grey seals	East Coast, Scotland	Liver	Below detection limits (<0.5)	1980
Grey seals	Farne Islands, North East England	Liver	Below detection limits	1978.
Grey seals	Tees Estuary	Liver	0.1	2000
Harbour seals	East coast, Scotland and England.	Liver	3.0–12.0	1975
Harbour seals	Dutch Wadden sea	Liver	<0.05-2.3	1979
		Kidney	0.16-0.23	
		Blubber	<0.05-1.0	
		Brain	<0.05-2.0	
		Spleen	0.16-0.40	
		Heart	0.29-0.61	
		Placenta	<0.05	
Harbour seals	German coastal waters	Liver	0.09-0.74	1972
		Kidney	0.08-0.60	
		Muscle	0.03-0.10	
Harbour seal	Tees Estuary	Liver	0.05	2003
		Kidney	0.06	
		Blubber	Not detected	
		Brain	0.04	

Table 6.17. Cadmium concentrations (mg kg^{-1} wet mass) in harbour seals from data in Thompson (1990) and present data from the Tees Estuary (2003).

Location	Age	Tissue	Concentration (mg kg^{-1} wet mass)	Date
Dutch Wadden sea	Adult	Liver	0.03-0.21	1979
		Kidney	0.15-0.17	
		Blubber	<0.01-0.02	
		Brain	<0.01-0.14	
		Spleen	0.04-0.09	
		Heart	0.06-0.47	
		Placenta	<0.01	
Dutch Wadden sea	Fetus	Liver	<0.24	
		Brain	<0.01	
Coastal waters off East Anglia and West Scotland		Liver	1.10	1976
		Kidney	1.9	
German coastal waters		Liver	0.01-0.21	1990
		Kidney	0.06-1.0	
		Muscle	0.002-0.08	
Tees Estuary	2 year old	Liver	0.02	2003
		Kidney	0.02	
		Blubber	Not detected	
		Brain	Not detected	

Table 6.18. Chromium concentrations (mg kg^{-1} wet mass) in harbour seals from data in Thompson (1990) and present data from the Tees Estuary (2003).

Location	Age	Tissue	Concentration (mg kg^{-1} wet mass)	Date
Dutch Wadden sea	Adult	Kidney	0.15-0.59	1979
		Heart	0.70-1.20	
		Spleen	0.80-1.40	
		Brain	<0.01-0.14	
		Blubber	1.0-2.8	
		Placenta	0.50	
Tees Estuary	2 year old	Liver	0.0	
		Kidney	0.06	
		Blubber	0.2	
		Brain	0.2	

Cr concentrations were lower than those reported in literature for most tissues, except in the brain where concentrations were significantly higher (Thompson, 1990).

Wilson (1994) measured Zn, Cu, Pb, Cd, Cr and Hg concentrations in the liver and sub-cutaneous blubber of three seal pups born on Seal Sands in 1989, 1991 and 1993 (Table 6.19). The pups had died between one to five days after their birth. The only detectable metal concentrations in the liver of the three seal pups were Zn, Cu and Hg but the concentrations were lower than those stated in the literature, as were the Hg concentrations in these three pups compared to the Hg concentrations measured in the liver of juvenile and subadult seals recovered from the Tees Estuary. Zn and Cu concentrations in the liver were comparable between the three pups and the juvenile and subadult seals.

Table 6.19. Metal concentrations measured in three harbour seal pups from Seal Sands, 1989 -1993

Seal	Date	Zinc (mg kg^{-1})	Copper (mg kg^{-1})	Mercury (mg kg^{-1})	Source of analysis
5 day old, male	July 1989	60	26	1	MAFF
1 day old, female	July 1991	112	8	0.5	AES
4 day old, male	June 1993	68	11	0.8	AES

Source of Analysis:
MAFF Ministry of Agriculture, Fisheries and Food
AES Analytical and Environmental Services

Marine mammals have developed mechanisms to regulate internal concentrations of essential trace elements so concentrations should reflect the maintenance range of between 20-100 mg kg^{-1} in the liver (Law *et al*, 1991; 1992). Zn concentrations for the liver of the juvenile, subadult and two pups fell within this maintenance range, whereas one pup born on Seal Sands in 1991 had Zn concentrations in the liver exceeding this maintenance range (112 mg kg^{-1}) (Wilson, 1994). This may have been due to measurement error but that is unlikely since the analysis is conducted by well trained personnel. There is a lack of agreement in the literature regarding whether Zn is transferred across the placenta or not. This pup either received Zn via the placenta, the milk or passively from the external environment after death. Cu concentrations in the livers of adult marine mammals tend to be within the range of 3 to 30 mg kg^{-1} (Law *et al*, 1991). Copper concentrations were within these maintenance levels for the

three seal pups born on Seal Sands. Copper is transplacental so the low Cu tissue levels in the pups indicate low Cu concentrations in the mothers. The Cu concentration in the adult grey seal liver collected from Seal Sands slightly exceeded the range of homeostatic regulation at 31.5 mg kg^{-1}.

Mercury concentrations of 500 mg kg^{-1} dry mass and 280 mg kg^{-1} dry mass recorded in the liver of the subadult grey seal and juvenile harbour seal, respectively were high. Recent research indicates that heavy metals like Hg, Cd and Pb can have endocrine disrupting effects in humans and rodents and this has been suggested to be a cause of the ongoing decline of harbour seals in Alaskan waters (Dehn *et al,* 2005). Cadmium and Pb concentrations in the two seals were low but the Hg concentrations were high. The concentrations may be higher than expected in healthy seals. It is also not known where these two seals originated from and the high levels may reflect Hg concentrations from a different location. Hg concentrations in the pups were 0.5 to 1 mg kg^{-1}, which are negligible. There is a lack of agreement in the literature regarding whether Hg is transferred across the placenta in significant amounts. If Hg does transfer across the placenta this suggests a low concentration of Hg in the mothers of this pup.

Metal concentrations in seals from the Tees Estuary were higher in the liver than the kidney, except for Cd and Cr concentrations. Most literature sources report that metal concentrations are higher in the liver and kidney than the brain and blubber in seals (Drescher, 1977; Oehme, 1978; Eisler, 1981; Thompson, 1990 and Bustamante *et al,* 2004). The liver is a site of detoxification and storage of metals and therefore generally the most important accumulator of metals in pinnipeds, followed by kidney levels. Generally, Cu, As and Cr concentrations in the brain of both seals collected from the Tees Estuary were higher than expected (Thompson, 1990), as were the concentrations of As, Cr and Hg in the blubber (André *et al,* 1991). Hg concentrations in the grey seal were higher in the blubber than the kidney (not measured in the brain) but in the harbour seal Hg concentrations were higher in the brain than the kidney and higher in the kidney than the blubber. The differences in metal concentrations between studies could reflect the type of food consumed, the quantity of food intake, pollution levels in different geographical regions, metabolic clearance and elimination of metals through

parturition and lactation. Alternatively, there may have been input or output of metal concentrations after death.

The metal burden of body organs for the subadult, grey seal and juvenile, harbour seal were highest in the liver and the blubber. This may be expected as they are the largest of the organs measured, particularly the blubber. The liver is the largest organ used for detoxification and hence the metal content would be expected to be high. The total metal content was particularly high for Hg suggesting that this metal had been biomagnified. Lead and Cd were low in all body components measured. Chromium concentrations were low in the body components, except liver. The low Pb content may be due to the fact that Pb tends to accumulate in bone (Law, 1995) and metal content in bone was not measured. Cadmium concentrations were low in the prey of the seals and so would be expected to be low in the predators. This suggests Cd did not biomagnify in these two seals.

Although the grey seal and the harbour seal used for analysing metal concentrations were found dead on Seal Sands this is not necessarily where they originated from and in fact, since grey seals do not breed at Seal Sands, the grey seal will not have originated from this area. The seals were found in the Tees Estuary however, and therefore will have taken in metal concentrations from local prey species. Transient species and dead bodies can float long distances so the origin and time of death is generally unknown. In addition, stress associated with injury or starvation before death can influence pollutant loads. Lead, for example may be released with calcium during stress because it follows calcium during bone formation (Thompson, 1990). There was a twofold difference in liver concentrations of Hg and Zn in northern gannets, *Morus bassanus* found dead on the east and west coast of Britain which was largely offset by a twofold difference in liver mass, reflecting the emaciated condition of the corpses from the west coast (Thompson, 1990). Metal concentrations may therefore be high in seals that have died from sickness due to emaciation and, in this case, the metal load in the liver would not be as high. The male grey seal analysed in this study had died of septicaemia but the cause of this was unknown. The female harbour seal had been found in a pipe leading onto the Brinefields from Greatham Creek. It was thought that she must have been trapped, although drowning was not the cause of death and she was not emaciated so she had

not starved. It was considered highly unlikely that she could have been washed into the pipe after death as more force would have been required. The cause of death of the female seal and the cause of the septicaemia of the male seal could not be established from the post mortem and may have affected metal concentrations.

The two seal samples were not directly comparable because they were different species, sex, age, and body size. The grey seal was recovered in August, whilst the harbour seal was recovered in February and it was not known whether either of the two seals had actually originated from the Tees Estuary or had inhabited Seal Sands. The three pups born in the Tees Estuary were suitable for comparison as they were monitored daily from birth (Table 6.19). They were known to have originated from the estuary, although their mothers will have come originally come from different locations (since the first birth of harbour seals recorded on Seals Sands since the nineteenth century was in 1989), and the time of death for the pups was known within one day.

6.5.2. Metal concentrations in the faeces of Tees Estuary seals

There was higher variation in the non-essential metal concentrations in seal faeces, than in the essential metals. This suggests a regulated absorption of essential metals compared to that of non-essential metals. Marine mammals have developed mechanisms to regulate internal concentrations of essential trace elements (Law *et al*, 1991; 1992).

For any given individual seal faeces, if one metal had a high concentration then all metals may be expected to have high concentrations. Conversely, in the case of one metal having a low concentration in a seal faecal sample then all the metals may be expected to have low concentrations. There was very highly significant negative correlation between Zn and Cd and very highly significant positive correlations between Cu and Pb, Cu and As, Cu and Cr, Pb and As, Pb and Cr and As and Cr. The significant negative correlation between Zn and Cd concentrations may suggest that one of the metals is inhibiting the uptake of the other metal. Teigen *et al* (1999) reported that Zn and Cd interacted in competitive binding.

All metal concentrations in faeces were higher in winter than in summer, except Zn. Cu, Pb and As concentrations, however, were not statistically significantly different. Lower metal content in faeces in the summer correspond with lower estimated daily metal burden taken in from prey consumed per seal during July to August for all metals, with the exception of As. The estimated daily As burden intake from prey was lowest in November to December, followed by July to August. This may explain why there was not a significant difference in As concentrations in seal faeces between summer and winter.

6.5.3. Metal budgets in the Tees estuary seals

The tissue burden of heavy metals in top predators reflects the balance between ingestion and elimination. Diet is considered the main metal uptake route for adult marine mammals (Law, 1995; Bustamante *et al,* 2004). Female adults may transfer metals to pups during the summer via transplacental transfer and milk (Laws *et al*, 1992). Law *et al* (1992) reported that transplacental transfer of Cu and Pb was relatively high in marine mammals, whereas that of Zn, Cd, As and Hg was relatively low, although some authors disagreed and stated that there was considerable transplacental transfer of Zn, Cd and Hg (Teigen *et al*, 1999; Dehn *et al*, 2005). Relatively high levels of Pb and Cd may be transferred via the milk (Law *et al*, 1992). The metal concentrations analysed in seal pups that were born on Seal Sands were relatively low, except for high Zn concentrations in one pup (Table 6.19). This suggests, for these three pups, that transplacental transfer of metals was not high except for the essential metal, zinc. The transfer of metals through the placenta and milk would only eliminate metals in a few individuals on Seal Sands due to the low reproductive rate.

The faeces are expected to be a major excretory route for metals ingested within the food and not absorbed across the gut wall. The water in seal urine is thought to be mostly, if not all, from the food rather than drinking water (Ronald *et al*, 1984). The urine could potentially be an important excretory route for metabolized metals, but it could not be measured in this study. Metal concentrations are also excreted via the hair of seals during the moult. Metal concentrations in the hair of the Tees seals could not be measured as it would have required capturing the seals and this was seen as too disturbing. In breeding females there may also be loss via transplacental transfer and milk production.

The estimated mean daily wet mass of prey calculated from prey remains in each seal faecal sample was between 0.05 kg in July to August to 0.6 kg in January to February. Norday and Blix (1988) calculated the food requirement of a harbour seal to be approximately 2.5 to 5% body mass per day (Norday and Blix, 1988). A 100 kg harbour seal will require approximately 2.5 to 5 kg of food daily. The estimates of wet mass consumed by harbour seals in the Tees Estuary are eight to 50 times less than the daily requirement quoted in the literature. The calculation of the mean daily wet mass is an under-estimate because it is based on the seals only voiding one faecal sample per day. Based on the difference between the wet mass of food required by harbour seals in the literature mean daily wet mass calculated for harbour seals from the Tees Estuary the seals excrete at least eight faeces daily.

The metal burdens and retention in seals are likely to be higher than reported in Table 6.11 and Table 6.12 since this is based on seals defaecating only once per day and faeces being the only excretory route. The retention will be at least eight times greater than quoted based on seals excreting at least eight faecal samples per day. Urine and hair are also potentially important output routes for metals in seals but the concentrations of urine and hair excreted have not been measured. The estimated retention will therefore be higher than quoted since it does not take into account these other excretory routes. In addition, the metal burdens in seals may also be under-estimated due to the erosion of otoliths recovered in the faeces leading to a low estimate of the biomass of prey consumed. The burdens of Zn, Cd and As taken in by seals from their diet were considerably higher than the metal content in the seal faeces and hence, the estimated retention of metals is relatively high. The burdens of Cu, Pb and Cr taken in by seals from their diet were high in relation to the metal content analysed in the seal faeces and hence, the estimated retention of metals were relatively low. A high proportion of the burden of Pb and Cd concentrations is expected to be output in the faeces as only about 5-6% of ingested Cd and <10% of ingested Pb is absorbed across the gut wall by mammals (Mason and MacDonald, 1986). This was not actually observed with similar Pb levels in the faecal samples to Cu and very low Cd levels.

It is expected that the metal burden in seals would be higher in the summer months as more pleuronectids and clupeids, with relatively high metal concentrations, were consumed than gadids. The estimated mean daily metal burden and hence, the retention of metals in seals however, was lowest in the July to August period for all metals. One reason for this may be because the seals are consuming more clupeids in the summer when they are most abundant into the Tees Estuary. Clupeids have fragile hard parts which are more likely to erode than more robust hard parts from species such as gadids and therefore the under-estimation of the biomass of prey species consumed is likely to be higher during the summer months. Low metal concentrations in a few faeces during summer may be due to transplacental transfer. The seals pup in late June to July so the low metal burdens would have be expected to occur in July to August if this had a significant affect, particularly after the lactation period. This however, would only affect the few female seals that gave birth. The moult season for harbour seals in the UK is late July to August (Anderson, 1990). If excretion of metals via hair was an important excretory route this would have been expected to have caused lower retention levels of metals after the summer.

The total metal burden in the two seals recovered from Seal Sands was estimated but since none of the organs were weighed the body composition of the seals had to be estimated from data for male and female Ross seals and crabeater seals (Bryden and Erickson, 1976) and three adult Weddell seals (Bryden *et al,* 1984). It is not known whether these species provide a representative body composition for harbour seals and grey seals and therefore this may have led to bias in the results. Metal concentrations were only analysed in the organs and blubber of the harbour seal and the grey seal so assumptions had to be made regarding the concentrations in the remainder of the body in order to estimate the total metal burden. The remainder of the body of crabeater and Ross seals was composed mainly of muscle (44 %), bone (10 %), skin (8 %) and blood (14 to 15 %) (Bryden and Erickson, 1976; Bryden *et al,* 1984). The assumption was made that metal concentrations in these body tissues would be similar to that in the heart (which is a vascularized muscle). The difference between burdens for each metal corresponded with the difference between the concentrations of each metal analysed in prey species for all metals, except for Pb. Nearly 90% of the total body burden of Pb in marine mammals was found in the bones (Law, 1995). The body burden of Pb

in the seals was therefore probably considerably under-estimated. In further studies, metal concentrations should be analysed in all the components of the seal body and the mass of each component should be measured to allow the total metal burden to be accurately calculated. The inclusion of metal burden in other body components, such as the skin, bone, blood and gonads, may have increased the total body burden. Care should be taken in making comparisons particularly as two seals is not a representative sample. The seals were probably living and feeding in the Tees Estuary and therefore give an estimate of metal uptake from local prey species.

The estimated total metal burden in the two seals and the range of estimated metal retained in seals compared in Table 6.13 should give similar values if they were accurately calculated. The estimated metal retention in the seals should be higher than reported in Table 6.13, based on seals excreting at least eight faecal samples per day. In addition, metals may be excreted by other routes which were not measured such as the urine or the hair. The estimated total burden of Zn, Cu, As and Cr in the two seals were considerably higher than the levels of estimated retention per seal daily. The estimated retention per seal daily should be multiplied by at least eight since seals are expected to excrete at least eight faeces per day. The estimated total burden of Zn, Cu and As was considerably higher in the grey seal and comparable in the harbour seal than the levels of estimated retention per seal daily when it was multiplied by eight. The estimated total burden of Cr was higher in both seals than the levels of estimated retention per seal daily when it was multiplied by eight. The estimated total burden of Pb in the two seals was lower than levels of estimated retention per seal daily when multiplied by eight. The metal burden of Pb is likely to be a considerable under-estimate as Law (1995) found nearly 90% of the total body burden of Pb in marine mammals to be found in the bones. Pb concentration was not measured in the bone of the seals from the Tees Estuary. The estimated total burden of Cd in the two seals was lower than Cd retained daily multiplied by eight. This is due to the very low concentrations of Cd in seal body tissues with concentrations not detected in most body tissues. The mercury retention in seals could not be calculated as mercury concentrations were only measured in two prey species. The estimated metal retention of Hg in seals per day could

not be calculated since the metal retention is based on the input from prey and output from faeces and Hg concentrations were only measured in two prey species.

The metal burden in the body tissues of two seals found dead in the Tees Estuary were made despite two seals not being representative of the population. In addition, assumptions were made regarding the weight of the body organs and metal concentrations in body tissues were the concentrations were not measured. The estimates made are only rough due to the assumptions made but they do provide an indication of which metals are high and whether certain metals may be of concern. Further analysis of metals of concern would therefore be necessary. A relatively high burden of Zn, Cu, As and Cr in seal body tissues was expected as they are essential metals and will be stored at closely regulated levels. The non-essential metals, Pb, Cd and Hg may bio-accumulate in seals to levels of concern unless they are expelled by a different excretory route or stored so they are not bioavailable. The estimated burden of Pb appears low but approximately 90% of Pb is stored in the bone of marine mammals (Law, 1995) and this has not been taken into account. Cadmium concentrations were not detected in most seal body tissues and detected concentrations were low so Cd concentrations are not expected to be harmful based on metal concentrations in these two seals. Mercury concentrations were not measured in the blubber of the harbour seal so the total burden is an under-estimate. Mercury burden in the grey seal are high and of concern. This calculation however, is only for one seal and further analysis is required on other individuals to determine whether Hg bioaccumulation in the top trophic organisms is of concern in the Tees Estuary.

CHAPTER 7. HEAVY METALS IN CORMORANTS FROM THE TEES ESTUARY

This chapter investigates the uptake of heavy metals via the diet by the top predators from the Tees Estuary, the cormorant, *Phalacrocorax carbo*. Metal intake from prey is estimated from the seasonal biomass of prey species consumed, calculated in Chapter 3, and the seasonal metal concentrations in these prey species, calculated in Chapter 4. No cormorant carcasses were recovered during this study so no comparison could be made between metal intake and metal concentrations in cormorant body tissues. The metal concentrations in excretion products were not measured so retention could not be estimated.

7.1. EFFECTS OF HEAVY METALS ON FISH-EATING BIRDS

Cormorants are potentially sensitive indicator species of marine pollutants (Furness and Greenwood, 1993). Some of the cormorants that roost in the Tees Estuary will be residents, whilst others are migratory. Migratory birds will acquire pollutant burdens over extensive areas whereas the residents would be more useful indicators of local pollution. Cormorants, as top predators, are vulnerable to biomagnification of pollutants (Vaneerden *et al*, 1995).

Mortality and morphological abnormalities are often the first toxic effects of heavy metals noted in seabirds but subtle reproductive effects are also important as they affect long-term population dynamics (Burger and Gochfeld, 1993). Ingested Cd, for example, can cause reduced testis weight, failure of spermatogenesis, reduced egg production and eggshell thinning. Ingested Pb can cause lowered egg production, lowered testis weight, reduced hatching rate, decreased sperm count, lowered chick growth and behavioural abnormalities of chicks. Behavioural abnormalities detected in the chicks of herring gulls, *Larus argentatus* and common terns, *Sterna hirundo* which had ingested Pb were reduced feeding, locomotor effects, depressed growth and Pb-induced delay in parental recognition, depth perception and thermoregulation (Burger and Gochfeld, 1985; 1988a,b).

Concentrations of the essential metals, copper (Cu) and zinc (Zn), in seabird tissues exhibit low variation with regard to location or species, suggesting that these metals are metabolically regulated (Thompson, 1990). Zinc concentrations in terns, dunlin, *Calidris alpine*, curlew, *Numenius arquata,* sandpiper, *Calidris ferruginea* and herring gull are relatively constant with

levels tending to be lower than 90 mg kg^{-1} wet mass in liver tissue (Nicholson, 1981; Maedgen *et al*, 1982; Blomqvist *et al*, 1987). Copper concentrations remain relatively constant with mean levels in the liver of around 6 mg kg^{-1} wet mass, few values exceed 10 mg kg^{-1}. Further evidence for the regulation of Cu and Zn is provided by relatively low intra-population variation (Anderlini *et al*, 1972; Nicholson, 1981). Levels of non-essential metals, in contrast, vary greatly both within and between species. Mean Pb concentrations tend to vary from <0.01 to 5.3 mg kg^{-1} in the liver and <0.01 to 2.1 mg kg^{-1} in the kidney of seabird species (Furness and Rainbow, 1990). Mean Cd concentrations vary widely between populations, from < 0.1 to 32 mg kg^{-1} in wet mass in liver and 1.5 to 138 mg kg^{-1} in the kidney in seabird species, including common terns (Custer *et al*, 1986; Muirhead and Furness, 1988). Murton *et al*, (1978) suggested that seabirds may have evolved some capacity to regulate tissue concentrations of Cd as a consequence of long-term exposure to high natural levels in oceanic food chains. Mercury and Cd levels in the tissues of seabirds from Gough Island in the South Atlantic Ocean appeared to be natural but they were greatly in excess of levels known to have severe toxic effects on rats and man, and associated with kidney lesions in seabirds from other locations (Muirhead and Furness, 1988). This suggests that these seabirds are able to tolerate high levels of these metals.

The antagonistic role of certain trace elements and the binding of metals to specific proteins may modify metal toxicity after chronic exposure and should be taken into account (Hutton, 1981). Positive correlations between Zn and Cd have been found in the kidney of seabirds, as have positive correlations of selenium and Hg in the kidney and liver (Hutton, 1981; Nicholson, 1981). The relationship between Zn and Cd is thought to involve elevated Zn concentrations inducing the formation of MT which then binds Cd, giving protection against Cd toxicity. Indication of the antagonistic interaction between Zn and Cd was found in shorebird species at Teesmouth in the 1970s (Evans and Moon, 1981). Species carrying the highest concentrations of Zn and Cd carried the lowest concentrations of Pb, and vice versa.

Heavy metal levels may vary between seabird species due to factors such as age, body size, feeding and migratory habits, moult strategy and taxonomic influences on physiology

(Walsh, 1990). Quirke (1995) compared heavy metal loads in the chicks of common terns in the Tees Estuary to a study of metal levels in adult common terns in Canada (Connors *et al*, 1975). The liver concentration was far greater in the chicks, whereas the kidneys and breast muscle in adult terns had higher Cu concentrations than in chicks. This may indicate that as terns get older they are able to regulate the Cu concentration in the liver but accumulate Cu in the muscle and kidney. Pb concentrations in the bone of adult common terns (Connors *et al*, 1975) were considerably higher than in the chicks, suggesting that Pb bioaccumulates with time (Quirke, 1995).

Cadmium was not detected in 24 of 38 samples of common tern chicks and there was no significant difference between Cd levels in young and old chicks. Cd concentrations in adult common terns (Connors *et al*, 1975) were much higher than in chicks (Quirke, 1995), suggesting that Cd bioaccumulates with time. Cadmium was found to accumulate with age in several studies comparing levels in chicks or juveniles with adults (Furness and Hutton, 1979; Hutton, 1981; Maegden *et al*, 1982; Blomquist *et al*, 1987, Saeki *et al*, 2000). Cormorant chicks had very low Cd concentrations, even in the liver and kidneys (Saeki *et al*, 2000), significantly lower than these tissues of juveniles which, in turn, were lower than those in adults. Cadmium exhibits age-related concentration increases but cormorants appear to be able to weakly regulate Cd by excretion through the renal system (Saeki *et al*, 2000). In addition, Cd binds strongly to MT, as does Zn, and this binding is thought to offer some protection against toxicity.

Mean Hg levels were significantly increased in cormorant chicks to adults in all tissues except the brain but in juveniles to adults the increase was only slight and not statistically significant (Saeki *et al*, 2000). This could be due to Hg being eliminated by moult from the feathers and therefore resulting in only a slight, non-significant Hg accumulation in adults. Other studies detected no significant difference of Hg levels with age. It may be that elimination of Hg into feathers during the moult (Furness and Rainbow, 1990), almost entirely as methylHg is efficient enough in these cases to prevent age accumulation of this particular toxic form. There is also evidence however, for demethylation of Hg by seabirds and subsequent storage of inorganic Hg in the liver. This suggests age accumulation of Hg

is likely where species have particularly high liver concentrations of inorganic Hg. Elliott and Griffiths (1986) studied Hg contamination in components of an estuarine ecosystem. Components were ranked according to their concentration factor and the highest biota concentration factor occurred in truly resident, estuarine, dermersal fish and wading birds. Mercury was shown to biomagnify along a direct consumer route where the consumer is a true estuarine resident or largely dependent on a single food source.

Gender differences in metal levels may occur in relation to sexual size dimorphism, different diet or lower levels in females due to sequestering of metals in eggs. Some metals interact with calcium and magnesium and are incorporated differently in the male and female of reproductive age (Burger and Gochfeld, 1993). Compared to levels of individual variation; however, gender variations in pollutant loads tend to be small. Only a few studies detected significant gender differences between metal levels in seabirds and waders. These studies, include higher levels of Cd in female bar-tailed-godwit, *Limosa lapponica* (Evans and Moon, 1981), higher levels of Pb but not Cd in female common terns (Burger and Gochfeld, 1991), higher Cd levels in male dunlin (Ferns and Anderson, 1994) and higher levels of Cd in female oystercatchers, *Haematopus ostralegus* (Hutton, 1981).

Seasonal changes in body mass can alter the perceived metal concentrations even though the total body pollutant load is unchanged. The influences of seasonal processes (namely breeding and moult) and seasonal dietary differences are causative factors in the changes in metal burdens. Other important influences include seasonal variations in fat or protein content of tissues or variations in enzyme activity (Bull *et al*, 1983). Stewart *et al* (1994) analysed seasonal variation in heavy metal levels in the tissues of common guillemot, *Uria aalge*. There was a strong seasonal fluctuation in Cd levels in the liver and kidney, rising significantly between April and June and declining again from June to November. These changes were apparent in both adult and juvenile birds. Seasonal variations in metal concentrations in the liver of seabirds with respect to moult cycles can be considerable (Thompson, 1990). In a study of shorebirds in Teesmouth seasonal levels of Hg in the liver varied in parallel with Zn and liver concentrations rose before the birds moulted into breeding plumage in the spring (Evans and Moon, 1981).

7.1.1. Tissue distribution of metal concentrations in fish-eating birds

Heavy metals tend to accumulate in specific tissues in seabirds. In ten seabird species, heavy metal levels in the liver averaged higher than in the kidney, while in five seabird species the reverse was true (Muirhead and Furness, 1988). Copper concentrations were very similar between the liver and kidney of seabirds from Gough Island in the South Atlantic Ocean (Muirhead and Furness, 1988). Lead levels accumulate in the bone, thus providing the best measure of chronic exposure (Scheuhammer, 1987) whilst soft tissues are better indicators of localised and short-term exposure. The avian kidney tends to accumulate higher Pb levels than other soft tissues but it has been suggested that the liver is the better short-term monitoring tissue of localised Pb levels and that increased liver/kidney ratios may indicate recent exposure (Bull *et al*, 1983). Levels of Pb in any of the soft tissues rarely exceed 1 mg kg^{-1} wet mass (Bull *et al*, 1977). Ninety percent of the total body burden of Cd is contained within the liver and kidneys (Scheuhammer, 1987), whereas Saeki *et al* (2000) found Cd levels in cormorants to predominately accumulate in the kidney (40%). The actual ratio of liver: kidney indicates exposure time. Liver/kidney ratios > 1 are indicative of acute exposure to high Cd levels (Scheuhammer, 1987). Brain, feather and muscle levels of Cd in cormorants were almost all below detection levels (Saeki, 2000), the former corresponding to the stated prevention of Cd transfer to the brain in animals by the blood-brain barrier (Yamamoto *et al*, 1987).

The distribution of Hg between tissues was investigated in black-headed gull chicks, *Larus ridibundus*, fed doses of methyl Hg (Lewis and Furness, 1991). Hg accumulated differently in the internal tissues, concentrations in the kidney exceeding those in the liver, which in turn exceeded those in the muscle. All feather types contained higher Hg concentrations than internal tissues. Skeletal muscle comprised the major storage site for Hg in the body because although concentrations were not as high as in the liver, kidney and feathers, the greater mass of muscle makes for greater total accumulation. The proportion of Hg deposited in kidney and primary feathers increased with dose whereas the opposite was found with the carcass. Hg appears to be deposited in the carcass when levels are low but deposited in the kidney and primary feathers at high Hg levels.

7.2. METAL INTAKE AND OUTPUT ROUTES IN FISH-EATING BIRDS

The tissue burden of contaminants, including heavy metals in top predators reflects the balance between ingestion and elimination. A simplified equation of uptake, retention and loss of heavy metals in top predators may be described as:

UPTAKE = LOSS + RETENTION

Birds accumulate heavy metals from their food and eliminate it via faeces, feathers and, in females, eggs (Lewis and Furness, 1991). Other excretion routes for cormorants are regurgitated pellets, urine (uric acid) and possibly, nasal secretion from the salt gland. The simplified equation of uptake, retention and loss of heavy metals in adult cormorants may be described as:

UPTAKE VIA FOOD = (EGESTION VIA FAECES, URIC ACID AND REGURGITANTS + EXCRETION VIA FEATHERS) + RETENTION

There is a dynamic balance between intake and elimination rates of food and metal burdens. Heavy metal budgets in common tern chicks were derived from intake and excretion rates and the storage of each metal (Quirke, 1995). Zn accumulated with increased age, although this was affected by individual variation and unknown wet mass at the time of death. A lower proportion of Pb was accumulated than Zn, with approximately 80% being excreted. There were errors however, in estimating intake and excretion rates which reduces the reliability of the metal budget calculations, as does individual variation in excretory efficiency and metal storage.

7.2.1. Metal intake by the diet

Diet was the main route of metal intake in experimental studies on non-seabird avian species (Scheuhammer, 1987). Tissue levels of Hg, Cd and Pb increased in direct proportion to dietary levels over a particular dose range (Scheuhammer, 1987). There was a lower ratio of tissue to dietary concentration at higher doses, so dose responses tended to be non-linear. Platteeuw *et al* (1995) found that individual variations in prey species composition, as well as individual differences in physical condition of the bird, produce differences in the contaminant load of the liver (on a lipid basis) of a factor of two to three in the cormorant. Increased breeding success

in cormorants can be ascribed to a shift in food choice towards prey with lower contaminant loads (Boudewijn and Dirksen, 1995).

The mean daily intake of eight breeding male and six breeding female cormorants with mean body mass of 3200 +/- 183 g and 2325 +/- 117 g, respectively, recorded over a total of 89 foraging trips were 828 +/- 271 g and 828 +/- 166 g, respectively (Grémillet, 1997). Predictive equations were used to estimate daily food intake for a wintering adult cormorant with a body mass of 2901 g at 843 g and estimated daily food intake for a wintering juvenile cormorant with a body mass of 2657 g at 790 g (Carss, 1997). Daily food intake estimated from daily energy expenditure of radio-tracked birds ranged from 248 to 415g for cormorants in summer (mean of 303 +/- 22, n = 3) and from 264 to 587g for cormorant in winter (mean of 401 +/- 19, n = 5) (Hughes *et al*, 1999).

7.2.2. Metal output by birds via feathers

Birds moult at least once a year and sequestering metals in inert plumage is a mechanism by which they can expel heavy metals (Furness and Greenwood, 1993). Some levels of metals are higher in feathers than in other tissues so a considerable amount can be eliminated since feathers account for approximately 5 to 12 % of the body mass of birds (Burger and Gochfeld, 1993).

Most seabirds are long-lived and even a modest retention of metals in the body through each moult could lead to accumulation with age (Burger *et al*, 1994). Burger *et al* (1994) examined the concentrations of heavy metals in the breast feathers of common terns. Concentrations of Cr increased significantly with age among adults (2-21 years old) whereas concentrations of Hg, Cd and Pb did not. Hg and Cr concentrations were higher in fledglings than adults, this probably reflected higher exposure to these metals in the breeding area than the winter quarters where the adult feathers grow.

The proportion of Pb body burden in the feathers has been reported as 40-60%, whereas Cd, Cu and Zn levels in feathers were less than 30% of the body burden (Burger and Gochfeld, 1993). Twelve to 25% of the body burden of Zn is incorporated into the feathers because it is an

essential component for feather formation. Generally, Hg levels are highest in the feathers and Hg excretion into the feathers at each moult is an efficient protective mechanism against continued accumulation in the body with age. It has been shown experimentally that Hg is incorporated in a dose-dependent fashion (Scheuhammer, 1987). Fish-eating birds ingest a high Hg intake and therefore Hg accumulation in other tissues exceeded elimination from the body through the feathers (Saeki *et al*, 2000). Cormorants tend to have high Hg levels, although it is variable and expected to be lower for birds frequently foraging inland. Saeki *et al* (2000) detected cormorant Hg levels in the feathers to be 40% of the total body burden. Almost 100% of the Hg in feathers is methylHg. Methyl Hg bonded into the feather keratin reflects the amount of Hg in the blood at the time when individual feather was formed and it will begin to accumulate in the body once moult is complete (Furness and Rainbow, 1990). There is considerable variation of Hg concentrations in feathers, however, between individual birds (Walsh, 1990).

Large samples of feathers can be collected to determine metal loads, without the death of the birds but there are a number of problems with measuring metal levels in feathers. Atmospheric pollutants may be deposited on the surface of feathers and mask endogenous levels from dietary intake or levels stored during moult (Burger and Gochfeld, 1993). Hg is an exception as there is little atmospheric deposition of Hg. Metal concentrations measured from feathers also vary seasonally with the stage of moult and, location and diet during moult (Hahn *et al*, 1993). The selection of the feather type or part to be monitored, whether to use live or dead birds, time of year, sample size and variations with sex, age and diet will all affect metal concentrations measured in feathers. Metal concentrations in the feathers of migratory species reflect exposure to different locations, in addition to mobilisation from metals stored in the body (Burger *et al*, 1994).

7.2.3. Metal intake by chicks and output by adult, female birds via eggs

The egg has a highly consistent composition and so it is easy to ensure that metal concentrations are measured within the same part of the egg, making comparisons between eggs reliable (Furness and Greenwood, 1993). There is little transfer of Cd or Pb to eggs (Hutton, 1981; Burger, 1988b). Pb levels in eggs are invariably low, often below detection

limits (Furness and Rainbow, 1990). Cu levels in eggs also tend to be low with means between 0.15 to 1.8 mg kg^{-1}, whereas mean Zn levels in seabird eggs range from 1.5 to 22 mg kg^{-1} (Furness and Rainbow, 1990). In contrast, common tern eggs at the Wilton colony in the Tees Estuary were found to have elevated levels of the four metals analysed, Zn, Pb, Cu and Cd (Quirke, 1995). Only deserted eggs were analysed however, and this may have biased the results. Eggs can indicate localised environmental exposure levels for Hg since dietary methylHg is dose-dependently transferred to the egg (Scheuhammer, 1987).

Removal of eggs to analyse metal concentrations is less problematic to the population than removing adults, especially if only one egg per clutch is removed. Deserted eggs can be analysed to avoid removal of healthy eggs from the population but they may not be representative of the population as the parents may have been young birds, poor quality birds or birds adversely affected by the toxins or the eggs may have become dehydrated or have bacterial infections. Eggs can be sampled from the same location each year, especially for colonial birds and are more likely to reflect metal uptake from local foraging than body tissues but metal concentrations can only be measured during the short period from laying to incubation. They therefore can not, be used to examine seasonal pollutant burdens and they only represent transfer of metal concentrations within a small, specific selection of the population that is breeding females. Heavy metal concentrations do not adequately reflect body burdens or dietary intakes of heavy metals and levels may vary through clutch sequence.

7.3. METHODOLOGY FOR ASSESSING METAL BUDGETS IN CORMORANTS

The mass of each prey species consumed by cormorants was estimated in Chapter 2 from the cormorant pellets. The mass of prey species consumed bi-monthly can be calculated from the proportions of each prey species consumed bi-monthly shown in Chapter 3. The mass of prey species consumed bi-monthly was then multiplied by the bi-monthly median metal concentrations for that prey species given in Chapter 4. The mass of Crustacea consumed could not be calculated from the prey remains in pellets so median metal concentrations in whole Crustacea were used.

The metal burden consumed daily by each individual cormorant was estimated. In captive trials cormorants produced one pellet per day independent of the number of meals or species of fish consumed (Zijlstra and Vaneerden, 1995). The total bi-monthly burden of metals was divided by the number of cormorant pellets collected bi-monthly that had prey present to estimate the metal burden taken in from the diet by each individual (Table 6.11).

Metal concentrations were not analysed in Chapter 4 for poor cod (69 consumed by cormorants), haddock (59 consumed by cormorants) and perch (115), roach (247) and wrasse (9) consumed by cormorants. It was assumed that metal concentrations in poor cod and haddock species would be similar to those in cod and therefore metal concentrations in cod were substituted for these species. Cormorant pellets containing perch, roach and wrasse (and any other fish present in that individual pellet) were eliminated from the analysis because metal concentrations had not been measured in these species during this study (Table 7.1). It was not known where the cormorants were feeding on these species and so they could not be collected from the cormorants feeding area for metal analysis. To obtain this information it would have been necessary to radio-track the birds.

Table 7.1. The bi-monthly number of cormorant pellets with prey present and not containing perch, roach or wrasse.

Month	No. of pellets with prey present	No. of pellets with prey present minus pellets with perch, roach and wrasse
Jan-Feb	58	53
Mar-Apr	59	52
May-June	60	55
Jul - Aug	59	54
Sept- Oct	60	60
Nov- Dec	60	55

7.4. RESULTS OF METAL BUDGETS IM CORMORANTS

The bi-monthly metal burden in cormorants from the Tees Estuary was estimated from the uptake and loss of metal concentrations. The bi-monthly uptake of metal concentrations by each cormorant was estimated from the biomass of each prey species present in pellets collected for each bi-monthly period and the median metal concentrations for each prey species. The biomass of fish consumed bi-monthly was estimated in Chapter 3, from the mass of fish consumed by each cormorant which was estimated in Chapter 2. The biomass of each prey species present in pellets collected for each bi-monthly period was multiplied by the bi-monthly median metal concentrations for that prey species, calculated in Chapter 4. The mass of Crustacea species could not be estimated from the prey remains in pellets. The median metal concentrations in whole Crustacea were used but this may be an over-estimate as the concentrations in exoskeleton that are bioavailable has not been tested as discussed in Chapter 5. Metal concentrations were not analysed in roach, perch or wrasse so if these species were present in a pellet then the contents of that pellet were not included in the calculation. The biomass and metal concentrations for each prey species were tabulated into a spreadsheet to calculate the metal burden for all cormorants in one given bi-monthly period. An example of this spreadsheet is given to calculate zinc burdens in cormorants during January to February (Table 7.2). The total metal burden is calculated for pellets collected in any bi-monthly period, excluding those containing roach, perch and wrasse. This total metal burden was divided by the number of pellets (52 pellets for the period January-February (Table 7.2)) analysed in each bi-monthly period to provide an estimate of daily metal burden for each individual cormorant.

Table 7.2. Example calculation of metal burdens in cormorants for the period of January to February

Prey species	Biomass (kg)	Median metal concentration (mg/kg)	Total metal burden for all cormorants (mg)	Total metal burden per cormorant (mg)
Cod	1.9	53.6 (52.1 -93.9)	101.9 (98.9 – 178.4)	2.0 (1.9 -3.4)
Whiting	5.8	64.5 (34.6-195.0)	374.1 (200.7-1131)	7.2 (3.9-21.8)
Saithe	1.8	50.4 (27.6 -56.6)	90.8 (49.7 -101.8)	1.7 (1.0 – 2.0)
Flounder	0.5	105.2 (47.8 -237.8)	52.6 (23.9 -118.9)	1.0 (0.5 -2.3)
Plaice	1.2	97.7 (54.5-128.2)	117.2 (65.4 -153.8)	2.3 (1.3 – 3.0)
0-1 year pleuronectids	1.0	114.4 (51.9 -258.4)	114.4 (51.9 – 258.4)	2.2 (1.0 – 5.0)
Common shrimp	0.04	125.0 (5.0 -270.0)	5.0 (0.2 -10.8)	0.1 (0.003 – 0.2)
Sandeel	0.03	123.3 (80.0 – 220.0)	3.7 (2.4 – 6.6)	0.07 (0.05 – 0.1)
Weever	0.8	55.9 (24.6 – 128.5)	44.7 (19.7 – 102.8)	0.9 (0.4 – 2.0)
Total			**904.4 (512.8- 2062.5)**	**17.5 (10.0 – 39.8)**

The bi-monthly median metal concentrations in the diet of cormorants and the prey composition in each pellet were used to calculate the metal burden per pellet for each bi-monthly period. Captive cormorants excrete one pellet daily so the metal burden in one pellet was used to estimate the daily metal burden consumed by an individual cormorant (Table 7.3). The estimated daily metal burden per individual cormorant varied considerably between bi-monthly periods.

Table 7.3. Estimated daily metal burden (mg) per individual cormorant for each bi-monthly period (minimum and maximum burdens in brackets).

	Zinc (mg)	Copper (mg)	Lead (mg)	Cadmium (mg)	Arsenic (mg)	Chromium (mg)
Jan-Feb	17.4	0.5	0.7	0.1	1.8	0.2
	(9.9-39.7)	(0.3 - 1.3)	(0.2 - 0.9)	(0.02-0.2)	(0.6-6.5)	(0.1-0.5)
Mar-Apr	40.0	1.1	1.3	0.1	4.4	0.5
	(21.9-82.2)	(0.5-2.4)	(0.3-3.3)	(0.03-0.3)	(1.4-10.9)	(0.3-1.4)
May-Jun	35.4	1.0	1.1	0.1	3.9	0.4
	(21.4-57.6)	(0.5-3.2)	(0.5-3.6)	(0.05-0.7)	(1.6-19.0)	(0.3-1.1)
Jul-Aug	22.8	1.6	0.9	0.1	2.4	0.4
	(14.3-39.5)	(0.3-2.8)	(0.3-3.5)	(0.04-0.5)	(0.8-12.1)	(0.1-0.9)
Sept-Oct	26.6	0.9	1.0	0.1	2.3	0.4
	(12.2-58.2)	(0.3-2.0)	(0.4-2.5)	(0.03-0.2)	(0.5-8.8)	(0.2-0.7)
Nov-Dec	24.9	0.7	1.1	0.1	2.8	0.4
	(14.4-74.6)	(0.3-8.8)	(0.4-3.6)	(0.02-0.3)	(0.6-12.3)	(0.2-0.9)

7.5. DISCUSSION OF HEAVY METAL BUDGETS IN CORMORANTS

The tissue burden of heavy metals in top predators reflects the balance between ingestion and elimination. Diet is considered the main metal uptake route for adult seabirds (Scheuhammer, 1987). Female adults may transfer metals to chicks during the summer via eggs (Furness and Rainbow, 1990). The concentrations of metals excreted via eggs by breeding females however, is relatively low for all metals, except Hg. Metal concentrations are also excreted via the feathers of birds during the moult. Seabirds may also excrete some metals via the salt glands. In breeding females there may also be a small amount lost via eggs. This is given here as a working hypothesis since retention was not estimated in this study.

In captive trials, cormorants produced one pellet per day independent of the number of meals or species of fish consumed (Zijlstra and Vaneerden, 1995) so the pellets collected are likely to be an accurate representation of the prey consumed by the cormorants in the Tees Estuary. The mean daily wet mass required by cormorants is 0.2 kg to 0.8 $^{+}$/- 0.3 kg (Grémillet, 1997; Hughes *et al*, 1999) and the estimated mean daily wet mass of prey consumed by cormorants in the Tees Estuary was within this range at between 0.3 kg and 0.4 kg, although the lower values of this range. This suggests that the cormorants in the Tees Estuary do not travel far to feed and therefore their energetic demand is within the lower values quoted for cormorants or the erosion of otoliths has led to the slight under-estimate of the mean daily wet mass of prey consumed by cormorants. It is expected that the metal burden in cormorants would be higher in the summer months as more pleuronectids and clupeids, with relatively high metal concentrations, were consumed than gadids but no clear seasonal pattern of metal burdens in cormorants was evident.

CHAPTER 8. GENERAL DISCUSSION AND CONCLUSIONS

As outlined in Chapter 1, the Tees Estuary supported a large diversity of estuarine species before the river banks were used for large scale human development. Today, the area is a large conurbation of towns with extensive industry. Despite the human presence and the loss of 90% of the mudflats, the area still supports a large number of moderately diverse species of estuarine invertebrates, which in turn provide winter feeding grounds for European and internationally designated populations of waders and waterfowl.

The Seal Sands mudflats were originally named due to the presence of a colony of over 1000 seals, which disappeared during the nineteenth century but recolonised with harbour seals, *Phoca vitulina* and smaller numbers of grey seals, *Halichoerus grypus* during the 1980s. The population size has gradually increased since. The harbour seals breed on the estuary but reproductive success has been limited. The maximum number of pups born in any one year between 1989 and 2003 was six in 2002 and during this period six pups died and four pups had to be rescued and rehabilitated to prevent them dying from starvation. The reproductive success is considerably less than the 20% of the population that Reijnders (1982) suggested was a normal pupping rate. Reproductive success in seals could be limited by the food supply. The gradual increase in seals joining the colony at Seal Sands from other locations however, suggests that the food supply is adequate and the environment suitable. The length of time that the seals spend resting also suggests that they have a plentiful, localized food supply. Signs of malnutrition in seals include a more elongate body and the bones of the hips become visible, whereas the Tees seals have been observed to have a rotund body shape with good blubber coverage. Disturbance may reduce the pupping rate in seals by reducing the resting time, using energy resources and decreasing the duration of suckle bouts which influence pup weaning mass, which in turn influences 1st year survivorship (Engelhard *et al,* 2001). Disturbance at Seal Sands was sporadic and infrequent, although relatively high in 1995. It is unlikely that disturbance was intensive enough to reduce population numbers or restrict pupping. Engelhard *et al* (2001) did not find a direct effect of human disturbance on the efficiency of lactation in elephant seal, *Mirounga leonine* pups. Many contaminants within the estuarine environment, including

several heavy metals, may accumulate in large, long-lived marine mammals (Laws, 1995). Several authors have found evidence of metals transferred between the mother and pup through the placenta or the milk (André *et al*, 1991; Law *et al*, 1992; Teigen *et al*, 1999; Dehn *et al*, 2005). Heavy metals are toxic at critical levels (Laws, 1995). The aim of this study was to estimate the heavy metal uptake via the diet by harbour seals inhabiting the Tees Estuary. The metal intake load can then be assessed to evaluate whether critical levels were exceeded and therefore could have an adverse impact on seal health and reproductive success.

Metal concentrations could not be measured directly in a large sample of harbour seals from the Tees Estuary because there were only three natural seal mortalities on Seal Sands during the study. One was a seal pup that was stillborn but it could not be recovered because the mother carried it in her mouth for a number of days. Metal concentrations were measured in the body tissues of the other two dead seals recovered from Seal Sands but these were not comparative because the seals were different species, sex and age (Chapter 6). One seal was a two year old, female harbour seal and the other was a four year old, male grey seal. The diet of these two seals and hence, metal intake is likely to vary because of these differences but both seals had empty stomachs on recovery so this could not be assessed. This study concentrates on harbour seals as Seal Sands is not suitable breeding habitat for grey seals. The metal concentrations in the body tissues of these two seals provide interesting data to explore but a much larger sample size of comparable subjects would be required for the data to be objectively tested. It would have been unethical to kill seals within this small, recovering colony for scientific purposes and capturing the seals for blood/biopsy sampling may have led the re-establishing seal colony to abandon the area. It was decided to use an indirect approach to estimate the metal intake by harbour seals and to compare this to metal intake by another top predator in the Tees Estuary, the cormorant, *Phalacrocorax carbo*. Bi-monthly mass of prey consumed by harbour seals and cormorants from the Tees Estuary was determined (Chapter 3), median bi-monthly metal concentrations in the main prey species were analysed (Chapter 4) and this data was multiplied to obtain an estimate of metal intake.

Analysis of skeletal remains in seal faeces and regurgitated matter of cormorants was considered the best available method to estimate the number of prey species consumed and the size of prey consumed (Arim and Naya, 2003). The most frequent hard parts recovered from seal faecal samples and cormorant pellets collected in the Tees Estuary were otoliths (Chapter 2). Other bones were used to corroborate identification and increased the estimated quantity of prey consumed by 3 % in the seal diet and 0.9 % in the cormorant diet. Species identification from otoliths is relatively easy and the range of species in the diet of piscivores can be determined. Prime and Hammond (1987) estimated digestive efficiency of grey seals from otoliths in faecal samples, with comparable results to published values and concluded that no major component of the diet was unrepresented by otoliths in grey seal faeces. Harbour seals consumed 15 fish species and two Crustacea species and cormorants consumed 28 fish species and two Crustacea species. The body length of cod, *Gadus morhua,* whiting, *Merlangius merlangus,* saithe, *Pollachius virens,* poor cod, *Trisopterus minustus,* pleuronectids and dragonets, *Callionymus lyra* consumed by both predators was comparable, whilst the seals consumed a greater length range of herring, *Clupea harengus* than cormorants (Figure 2.3). Frequency charts of the biomass of main prey species consumed by seals and cormorants indicated that these predators most frequently prey on smaller individuals (Figures 2.6-2.8).

The use of otoliths to determine piscivorous diet may however, result in an under-estimation of the number of prey consumed (Jobling and Breiby, 1986) and the size of the prey consumed (Tollit, 1996). Some authors have used captive seals to estimate species-specific correction factors to account for the erosion of otoliths and to provide a more accurate number and size of species consumed (Da Silva and Neilson, 1985; Prime and Hammond, 1985; Prime and Hammond, 1987; Harvey, 1989; Cottrell, 1996; Tollit *et al,* 1997b; Marcus *et al,* 1998; Bowen, 2000). The estimated CFs however, is dependent on the individual captive seals that it is based upon. A number of species-specific CFs have been published without agreement as to which should be applied and CFs are expected to vary with region and season to some extent. CFs were not applied in this study due to their

limitations so the number and the size of prey consumed will be minimal estimates because the erosion and partial digestion of otoliths was not corrected.

Harbour seal faecal samples were collected from Greatham Creek, a tributary of the Tees Estuary (Appendix C). The faecal samples may have contained disproportionately more estuarine species than the seals were actually consuming. The main seal haul out is on the Seal Sands mudflats at the mouth of the estuary (Appendix C) but faecal samples were not collected from Seal Sands because grey seals also haul out in this area and faecal samples from the two species could not be distinguished. In addition, the mudflats quickly immersed requiring samples to be collected before the seals left naturally and hence, would be disturbing and the mudflats are very soft making access to humans dangerous. Radio-tracking could have been used to determine the main foraging areas used by the seals but this would have required capturing the seals which was potentially disturbing and only a limited number of individuals could be observed. Cormorants come inshore to roost at the Phillips Jetty on Seal Sands and regurgitate their daily food intake each evening (Zijlstra and Vaneerden, 1995). Pellets therefore provide an accurate sample of prey species consumed by cormorants from the Tees Estuary.

The diet of harbour seals and cormorants was compared in Chapter 3. Cormorants predated on a greater range of species, including two freshwater species. There was partial overlap of diet between the two predators with a similar prey profile of two dominant family groups, gadids and pleuronectids, being consumed. Direct competition was avoided by consuming different proportions and prey species and consuming other species that were less frequent or not present in the diet of the other predator. Clupeids were the most dominant prey species in the Tees Estuary (Table 1.7) with the highest numbers occurring during their summer migration (Figure 3.4 and 3.5) but they were only ranked as the 6th and 7th main prey items in the harbour seal diet and herring, *Clupea harengus* ranked as the 14th main prey item in the cormorant diet, whereas sprat did not rank in the cormorant diet at all. Herring were most frequently consumed by seals in July to August and sprat, *Sprattus sprattus* were most frequently consumed by seals in July to October, although a greater

mass of herring were consumed by seals in May to June and a greater mass of sprat were consumed by seals in January to February. Cormorants tend to prefer benthic prey (Leopold *et al*, 1998; Goutner *et al*, 1997). Whiting were the 3rd most frequent species in the counts from the Hartlepool Power Station intake water and ranked as the 3rd main prey item in the seal diet and the 2nd main prey item in the cormorant diet. Whiting numbers peaked in the counts from the Hartlepool Power Station and the seasonal consumption by cormorants during the winter months but they were most frequently consumed by seals in March to April (Figure 3.6). The counts of pleuronectids in the Hartlepool Power Station intake water were expected to be under-estimates as they are benthic fish so less likely to be swept in but the counts showed them to be the third most numerous family group after clupeids and gadids. Pleuronectids ranked as the 4th main prey item in the seal diet and the main prey item in the cormorant diet The number of flounder, *Platichthys flesus* in the counts from the Hartlepool Power Station peaked twice in June to July and September to October, whereas they were most frequently consumed by cormorants in July to October and were most frequently consumed by seals in July to February, peaking in November to December.

Concurrent with the dietary studies, was analysis of zinc (Zn), copper (Cu), lead (Pb), cadmium (Cd), arsenic (As) and chromium (Cr) concentrations in the main prey species (Chapter 4 and 5). To provide an estimate heavy metal uptake by predators the heavy metal concentrations were analysed in the whole body of the prey. The differences in metal concentrations between species, size and season were assessed to achieve as accurate an estimate of metal uptake as possible. The range of prey sizes collected from the Hartlepool Power Station intake water was smaller however, than the range of prey sizes consumed by harbour seals and cormorants from the Tees Estuary (Chapter2) and so the correlation between body size and metal concentrations could not be compared for the full range of prey body sizes. There were significant differences of metal concentrations between and within Crustacea and fish species. The highest metal concentrations generally occurred in Crustacea and pleuronectids and the lowest metal concentrations in gadids, although there were exceptions. Multivariate analysis also showed higher metal concentrations in Crustacea than fish for all metals, except zinc. The differences in metal concentrations between fish species were apparent for arsenic and chromium concentrations in plaice, *Pleuronectes platessa* indicating that plaice bioaccumulate these

metals to a greater extent than other fish species. Benthic organisms are expected to have higher metal concentrations in their body tissues because they are exposed to higher metal content in the sediment.

There were negative correlations between body size and metal concentrations but the majority of the correlations were not strong and may be influenced by Type I errors because of the large numbers of fish sampled. There were strong negative correlations however, between body size and chromium in plaice and cod. The highest metal concentrations measured in the study were found in some of the small individuals analysed, whilst lower metal concentrations occurred in larger individuals but there was also a range of metal concentrations measured in all but the largest of individuals. Seasonal metal concentrations were assessed and there were some metal concentrations were higher in summer for most species. The difference in metal concentrations between Crustacea and fish species and season were used to estimate metal uptake by their predators in Chapter 6 but the metal concentration in a prey species of a given size could not be predicted because the relationship between body size and metal concentrations was not strong enough for regression analysis to be conducted.

Metal concentrations were measured in common shrimp, *Crangon crangon,* flounder and sprat from the Tees Estuary in 1998-2002 by the Environment Agency (EA) (Tables 4.9 and 4.10). The EA only sampled a few individuals during the summer months but the samples were taken during a similar time period and from the same location so they were considered comparable. Metal concentrations in the shrimp and fish were either similar between the EA study and the present study or the range of metal concentrations in the present study was wider than the range given by the EA. Only the maximum Cr concentrations given for sprat in the EA study were considerably higher than those given in the present study. Cd concentrations were not reported in the flounder by the EA and hence could not be compared. The larger range of metal concentrations in common shrimp, flounder and sprat measured in this study compared to the EA study may be expected due to a larger sample size but the maximum values of Pb, Cd and As in fish and Crustacea and Cr in Crustacea were higher than those acceptable in the muscle for human consumption and may therefore be detrimental to predators. Predators consuming a diet of Crustacea and fish will have a

high metal intake, but it has been shown that organisms exposed to high metal levels for long periods often develop immunity (Dallinger *et al*, 1987). Additionally, the acceptable metal concentrations for humans are based on concentrations in the muscle, whereas the concentrations measured in this study and the EA study were for whole fish. Metal concentrations in fish muscle, and also liver and gill are given in Chapter 5 to enable metal concentrations in fish from the Tees Estuary to be compared with those from other studies.

The number of analyses that could be conducted for mercury (Hg) was limited by cost so analysis was only conducted on the two predominant fish species in the seal diet, whiting and flounder. Hg concentrations were below the limits of detection (1 mg kg^{-1} dry mass) in all flounder and 92 out of 101 whiting. Hg concentrations in the nine remaining whiting samples were detected at 2 mg kg^{-1} dry mass (Table 4.8). In retrospect, higher precision equipment should have been used but this was not available due to cost.

Trends in metal concentrations in the field between and within species are confounded by the range of influencing physiological and environmental factors. It is imperative to take into account the specific ecological situation of a given environment when investigating heavy metal pollution in fish (Dallinger *et al*, 1987). The ecology of the Crustacea and fish species in the Tees estuary, particularly regarding their diet and migratory movements, requires investigation to aid in the understanding of variation in metal concentrations in the biota. Measurements of environmental factors, such as salinity and the routes and rates of metal discharge to the Tees estuary would also aid in the understanding of variation in metal concentrations in the biota. In further studies, the sex and reproductive stage of the Crustacea and fish should be recorded, as should seasonal body composition and ecdysis in Crustacea. The body composition of fats, protein and water were not recorded. Metal distribution to body tissues may be influenced by fat content. Metals that have been biotransformed, such as tetramethyl Pb and methyl Hg, are non-polar and so tend to accumulate within fat rich tissues. Individual and seasonal variation in fat content in the liver can occur (Grimas *et al*, 1985) and fat content should be measured to control against variation.

Seals may not consume the exoskeleton of large Crustacea and even if the exoskeleton is consumed by the predator it may not be digested (Rainbow, P., Department of Biology, Queen Mary and Westfield College, University of London, pers. comm.) and hence, metal concentrations stored in the exoskeleton may not be bioavailable. Metal concentrations in the exoskeleton and the soft body tissues of Crustacea were assessed in Chapter 5. Higher Zn, Cu, Pb, Cd and Cr concentrations were found in the exoskeleton of the common shrimp compared to the soft parts and higher Pb, Cd and Cr concentrations were found in the exoskeleton of the shore crab, *Carcinus maenas* than in the soft parts. The availability of metal concentrations for predators may therefore have been over-estimated if metal concentrations stored in the exoskeleton are not bioavailable to predators. An over-estimate of metal intake due to metal concentrations in the exoskeleton not being bioavailable would be highest in May to June for harbour seals because they consume the largest number of shore crab during this period and March to April and July to October for cormorants because they consume the largest number of common shrimp at this time. Shore crab are the 5th most frequently consumed prey item in harbour seal diet and common shrimp are the 15th most frequently consumed prey item in cormorant diet. The bio-availability of metal concentrations stored in the exoskeleton of Crustacea for predators requires further study.

Partitioning of Zn, Cu, Pb and Cd between liver, muscle and gill in whiting, flounder and herring were assessed in Chapter 5. Most literature reports metal concentrations in fish tissues rather than whole body loads so this allowed further comparisons in metal concentrations in fish from the Tees Estuary to other studies. It was found that metal concentrations in fish were higher in liver and lowest in the muscle, corresponding with other literature (Hardisty *et al*, 1974 a,b; Wharfe and Van Den Broek, 1977; Marcovecchio *et al*, 1988; Huckle and Millburn, 1990; Henry *et al*, 2004). Zinc concentrations were highest in the flounder, Cu concentrations were highest in the flounder and herring and Pb and Cd concentrations were highest in the herring. Zinc, Cu and Pb concentrations were compared with concentrations measured in whiting and flounder muscle from the Tees Estuary by the EA, 1998-2002 (Table 5.18). The range of metal concentrations measured in whiting and flounder muscle were similar between both studies as expected when the fish were collected from the same location during the same time period. The concentrations in the fish muscle were not high

in relation to acceptable levels for human consumption but the National Monitoring Programme Survey of the Quality of UK Coastal Waters (MPMMG, 1998) indicated that Pb in flounder liver from the Tees Estuary may be high compared to other UK coastal waters. Cadmium concentrations in liver were relatively low compared to the most polluted UK coastal areas.

The bi-monthly biomass of prey species consumed by harbour seals and cormorants in the Tees Estuary assessed in Chapter 3 were multiplied by the bi-monthly metal concentrations in these prey species determined in Chapter 4 to give an estimate of the bi-monthly metal uptake from the diet by these predators (Chapter 6). Diet was considered the main metal uptake route for adult predators (Scheuhammer, 1987; Law, 1995; Bustamante *et al,* 2004). Median daily metal uptake from diet was calculated by dividing the bi-monthly metal uptake by the number of pellets or faecal samples analysed for that period. The estimated daily metal uptake per individual seal was highest during the winter months and considerably lower in July to August. This may have been due to an under-estimate of prey species consumed during the summer months.

The daily metal intake by harbour seals was based on the seals only defaecating once a day. The estimate of median daily wet mass of prey consumed by harbour seals in the Tees Estuary were eight to 50 times less than the daily requirement quoted by Norday and Blix (1988). This under-estimate of median daily wet mass was likely to be, at least in part, due to seals voiding more than one faecal sample per day. Based on the difference between the wet mass of food expected to be consumed daily by harbour seals based the energetic requirement given by Norday and Blix (1988) and the median daily wet mass estimated for harbour seals from the Tees Estuary, the seals are estimated to excrete at least eight faeces daily. The estimated median daily metal uptake by seals was recalculated on the basis that seals defaecate eight times per day (Table 8.1).

Table 8.1. Estimated median daily metal uptake (mg) per seal for each bi-monthly period (minimum and maximum burdens in brackets).

	Zn (mg)	Cu (mg)	Pb (mg)	Cd (mg)	As (mg)	Cr (mg)
Jan-Feb	262.4	12.8	15.2	1.6	40.8	4.0
Mar-Apr	127.2	4.0	4.0	0.8	23.2	1.6
May-Jun	224.4	6.4	5.6	0.8	24.8	4.0
Jul-Aug	34.4	1.6	1.2	0.2	4.8	0.6
Sept-Oct	144.8	4.0	4.8	0.4	12.0	1.6
Nov-Dec	146.4	4.0	6.4	0.4	14.4	2.4

The measured mean output of metals in the faeces should also be multiplied by eight to give an estimated daily metal output by faeces. The estimated seasonal metal retention per seal calculated from the estimated daily metal burden taken in by seals from their diet minus the measured mean output of metals in the faeces was recalculated to take into account seals defaecating eight times a day. The range of estimated daily metal retention values were compared with estimated total burden of metals measured in two seals recovered from Seal Sands (Table 8.2). In the female, harbour seal, the estimated daily retention of Zn, Cu and As were comparable to estimated burdens, whereas the estimated daily retention of Pb and Cd were larger than the estimated burdens and the estimated daily retention of Cr were lower than estimated burdens. In the male grey seal the estimated daily retention of Zn, Cu, As and Cr were lower than estimated burdens, whereas the estimated daily retention of Pb and Cd were comparable with the estimated burdens.

Table 8.2. Estimated total metal burden (mg) in two seals recovered from Seal Sands compared with the range of estimated daily metal retention values (mg)

Seal	Organ	Zn	Cu	Pb	Cd	As	Cr
	Male grey seal	**722.5-901.2**	**48.2-67.4**	**6.8-8.4**	**0.2-0.4**	**73.3-97.0**	**9.0-12.7**
	Female harbour seal	**198.8-246.2**	**12.1-16.4**	**1.8-2.2**	**0.006-0.007**	**16.4-69.0**	**3.5-4.6**
Estimated metal retention (mg) / seal daily		**16.0 – 242.4**	**-0.1 – 11.2**	**-0.4 – 12.8**	**0.2-1.6**	**4.5 – 40.6**	**-1.0 – 2.4**

This suggests that Zn, Cu, Pb, Cd, As and Cr have not bioaccumulated in these two seals. The estimated daily metal retention and estimated body burdens in the two seals are only rough estimates however, and limitations of the calculations must be considered. The

estimated daily metal retention is based on the bi-monthly mass of prey consumed estimated from hard part recovery in seal faecal samples. Hard parts in the faecal samples exhibit species-specific digestion or partial erosion leading to miscalculation of the number of prey consumed or the size of prey consumed. The re-calculated estimated daily retention is based seals defaecating eight times a day but this is a rough estimate and the precise figure is unknown and likely to vary between individuals.

The total metal burden in the two seals recovered dead from Seal Sands was estimated because the organs were not weighed so the body composition of the seals were estimated from data for Ross seals, *Ommatophoca rossi* and crabeater seals, *Lobodon carcinophagus* (Bryden and Erickson, 1976) and three adult Weddell seals, *Leptonychotes weddelli* (Bryden *et al,* 1984). Metal concentrations were only analysed in the organs and blubber so the assumption was made that the remainder of the body, mainly composed of muscle, bone, skin and blood, would have similar metal concentrations to that in the heart (which is a vascularized muscle). Heavy metal concentrations tend to be low in the muscle of seals (Dehn *et al,* 2006), so this assumption may have led to an under-estimate of the total metal burden in seals, particularly for Pb. Nearly 90% of the total body burden of Pb in marine mammals was found in the bones (Law, 1995). In future studies, metal concentrations should be analysed in all the components of the seal body and the mass of each component should be measured to allow the total metal burden to be accurately calculated. The metal burden in other body components, such as the skin, bone, blood and gonads, should be included in the calculation.

The daily burden of Hg in the prey species consumed by seals could not be calculated as concentrations were only measured in whiting and flounder and were below the detection limit in the majority of the small sample analysed. Mercury concentrations measured in the liver and blubber of the grey seal and the liver, kidney and brain of the harbour seal found dead on Seal Sands were high. This indicates that further research should be conducted on Hg concentrations. Heavy metals, such as Zn, Cu, As and Cr are essential in trace amounts for the health and growth of animals, including seals and concentrations in the prey would have to be excessive to have an adverse affect on the seals (Law, 1995; Bustamante *et al,* 2004). In contrast, no vital function has been found for Pb, Cd or for mercury (Hg) and mechanisms for

the internal regulation of these metals or to mitigate their toxic effects are less well developed, compared to those for essential metals (Law, 1995). Some research has found evidence of bioaccumulation of Cd and Hg (Koeman *et al*, 1973; Roberts *et al*, 1976; Drescher *et al*, 1977; Reijnders, 1980; Dehn *et al,* 2005) and therefore concentrations in predators may be high even if they are low in the estuarine environment and prey species. Thompson (1990) reported that Hg was the only metal that appeared to biomagnify and recent research indicates that heavy metals like Hg, Cd and Pb can have endocrine-disrupting effects in humans and rodents and this has been suggested to be a cause of the ongoing decline of harbour seals in Alaskan waters (Dehn *et al,* 2005).

Organochlorines (OCs) may be the cause of the low reproductive success of harbour seals in the Tees Estuary. They are lipophilic and persistent so high levels accumulate in marine mammals because of their high lipid content (Jenssen et al, 1996). Low to moderate body burdens of Polychlorinated Biphenyls (PCBs) were probably the cause of alterations in hormone levels of harbour seals and grey seals and high body burdens of PCBs were suspected to be the cause of reproductive failure in harbour seals in the Wadden sea (Reijnders, 1986) and grey seals in the Baltic sea (Helle et al, 1976). PCBs can cause reproductive impairment by altering menstrual cycle, embryo absorption, abortion, still births and impaired growth and survival of young. The analysis of organochlorines is very expensive however, and the necessary facilities were not available at Durham University. Collaborative work was attempted with CEFAS and Kingston-upon-Thames University. Fish samples, seal body tissues and also common tern eggs and chicks were supplied to the CEFAS laboratory in Burnham-on-Crouch and seal faecal samples were supplied for a research programme at Kingston-upon-Thames University but the analysis was not conducted due to funding cuts.

The estimated metal uptake via diet for cormorants from the Tees Estuary given in Chapter 7 are based on cormorants producing one pellet per day and this is corroborated by captive trials where cormorants produced one pellet per day independent of the number of meals or species of fish consumed (Zijlstra and Vaneerden, 1995). No cormorant carcasses were found during the study and it was not deemed acceptable to kill birds for the purposes of this study so total metal body burdens in cormorants could not be assessed. Originally

cormorant faecal samples and pellets were to be collected for metal analysis on a tarpaulin under their roosting site on the Phillips Jetty but this was not allowed for health and safety reasons. The mean daily wet mass of food required by cormorants is approximately 0.2 kg to 0.8 $^{+}$/- 0.3 kg (Grémillet, 1997; Hughes *et al*, 1999). The estimated mean daily wet mass of prey consumed by cormorants in the Tees Estuary was at the lower end of this range at between 0.3 kg and 0.4 kg. This indicates that the estimate of prey consumed may be a slight under-estimate or cormorants in the Tees Estuary have a low energetic demand. As the estimate of prey consumed is within the range of expected mean daily wet mass of prey consumed then the estimate of metal intake is also expected to be relatively accurate for cormorants, although it may be slightly under-estimated.

The results of this study suggest that while metal concentrations in Crustacea and fish species from the Tees Estuary are higher than in pristine estuaries, the metal uptake by the Tees seals is low and therefore metal content is not expected to have adverse effects. Seal carcasses should be analysed wherever possible to confirm these results and to analyse Hg and organochlorine concentrations. Median metal concentrations in Crustacea and fish were not of concern but maximum metal concentrations in some individuals suggested that hot spots still exist in the Tees Estuary. The accumulation of pollutants at higher trophic levels may be influenced by physicochemical properties of compounds and the physiological state of the animals on the rate of accumulation. Additionally, the geographical range over which predators feed can have a large influence on their pollutant body burdens as can the opportunistic feeding behaviour adopted by harbour seals and cormorants as these factors are likely to exert seasonal differences on the uptake of contaminants. A predictive equation to determine metal burden in top predators requires the measurement of metal output via alternative routes, including urine, feathers, hair, milk and eggs and also to measure metal concentrations in mothers and pups to determine transfer via the placenta and milk.

This study was funded by the Industry and Nature Conservation Association (INCA) in Teesside. INCA manage the Tees Seals Research Programme and this study has provided them with information regarding the seals diet. INCA have studied the affect of the seals on the Atlantic salmon, *Salmo salar* population as it migrates through the Tees Estuary via the Tees

Barrage. There was no evidence to suggest that any of the seals hauling out at Greatham Creek had consumed salmon. Salmon hard parts are very fragile and easily eroded but it would be expected that some bones would have been found if salmon were consumed. This study has also provided INCA with the indication that metal concentrations in the Tees Estuary are not having adverse affects on the top trophic levels. This not only helps them to understand affects on the seals but also provides data for Environmental Impact Assessments conducted for industries discharging metals into the Tees Estuary. INCA were commissioned to produce a report 'The State of the Natural Tees' (SONET) in 1996 and are now up-dating this report. The diet of the Tees seals and metal concentrations in Crustacea, fish and seals in the Tees Estuary will be included in the report. This study has also highlighted the need to further study mercury and organochlorines levels in the Tees Estuary and assess their affect on the biota of the Tees Estuary, including the Tees seals.

Seals and cormorants are frequently blamed for depleting stocks of commercial fish. The analysis of diet of the harbour seals and cormorants in the Tees Estuary has shown that both predators predominately consume gadids and pleuronectids but they also consume fish of low commercial value such as weever and dragonet. The metal concentrations analysed in Crustacea and fish in the Tees Estuary can be compared with concentrations measured in comparable biota from other estuaries to determine the affect of metals within estuarine food chains in British estuaries, including the potential affect to humans.

CONCLUSION

The aim of this study was to estimate metal intake by harbour seals and cormorants from the Tees Estuary via their diet. This was conducted by multiplying the bi-monthly biomass of prey species by median bi-monthly metal concentrations. The diet and metal uptake by seals and cormorants was compared.

Cormorants consumed 28 fish species and two Crustacea species and therefore exhibited a greater niche breadth of diet than the harbour seals consuming 15 fish species and two Crustacea species. There was partial overlap of prey species consumed with a similar prey profile of two dominant family groups, gadids and pleuronectids, consumed by both predators. Different proportions and sizes of main species were consumed by each predator and other species were consumed that were not common in the diet of the other predator. The biomass of prey consumed by seals and cormorants was significantly between bi-monthly periods. Seals consumed the highest biomass in January to February and May to June and the lowest biomass in July to August. Cormorants consumed the highest biomass of prey in November to April and the lowest biomass of prey in the summer months. Bi-monthly biomass of prey species was therefore used to calculate metal uptake.

Metal concentrations were significantly different between Crustacea and fish species. Multivariate analysis indicated that Cu, Pb, Cd, As and Cr concentrations were mainly different between Crustacea and fish species with Crustacea being the main accumulators. Plaice exhibited more accumulation of As and Cr than other fish species. Some metal concentrations were significantly higher in the summer for most species. The negative correlations between body size and metal concentrations were only strong for chromium in plaice and cod and hence, regression analysis could not be used to allow predictions of metal concentrations by body size to be made. Median bi-monthly metal concentrations for each prey species were used to estimate metal uptake by predators. Metal concentrations in the exoskeleton of Crustacea may not be bioavailable to predators and so the metal uptake may be over-estimated, particularly in May to June for harbour seals and March to April and July to October for cormorants because they consume the largest number of Crustacea at this time.

The estimation of the daily uptake of metals by predators is likely to be a minimal estimate as it is based on the biomass of prey consumed which was calculated from the presence of hard parts in excretory products and the hard parts may exhibit species-specific erosion during digestion. The biomass of prey consumed by cormorants, however does fall at the lower end of the range of expected prey mass consumed indicating that the estimate biomass of prey consumed may be relatively accurate of the cormorants in the Tees Estuary have a low energetic demand or a slight under-estimate. The estimated daily uptake of metals by cormorants will in turn therefore be a relatively accurate estimate or a slight under-estimate. The estimate of prey mass consumed by seals, and hence the estimated daily uptake of metals, may be less accurate than in cormorants because the hard parts may erode more in the digestive system of seals than those regurgitated by cormorants and whilst cormorants regurgitate one pellet per day it is not known how many faecal samples seals produce per day making the estimate of daily prey consumed from hard parts tentative. The estimated daily metal uptake per individual seal was highest during the winter months and considerably lower in July to August. This may have been due to an under-estimate of prey species consumed during the summer months. The estimated daily metal uptake per individual cormorant varied between bi-monthly periods.

The diet of seals and cormorants from the Tees Estuary can be used in a wider context to determine the impact of these predators on commercial fish. The metal concentrations in the biota of the Tees Estuary can be used as a bio-indicator of the metal concentrations and indicates that metals are not having a toxic effect on the top levels of the estuarine food chain.

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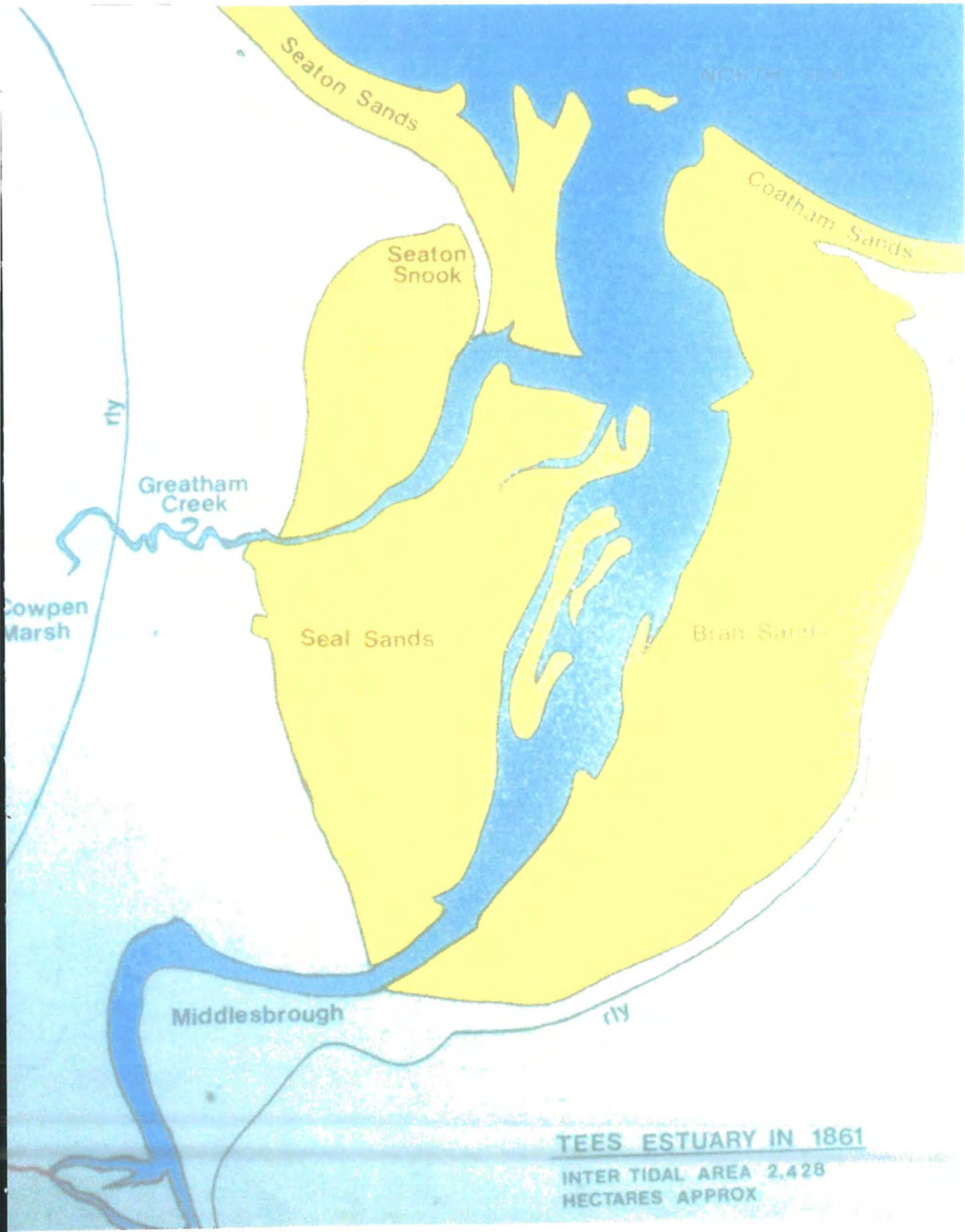
Appendix A.

Intertidal mudflats of the Tees Estuary

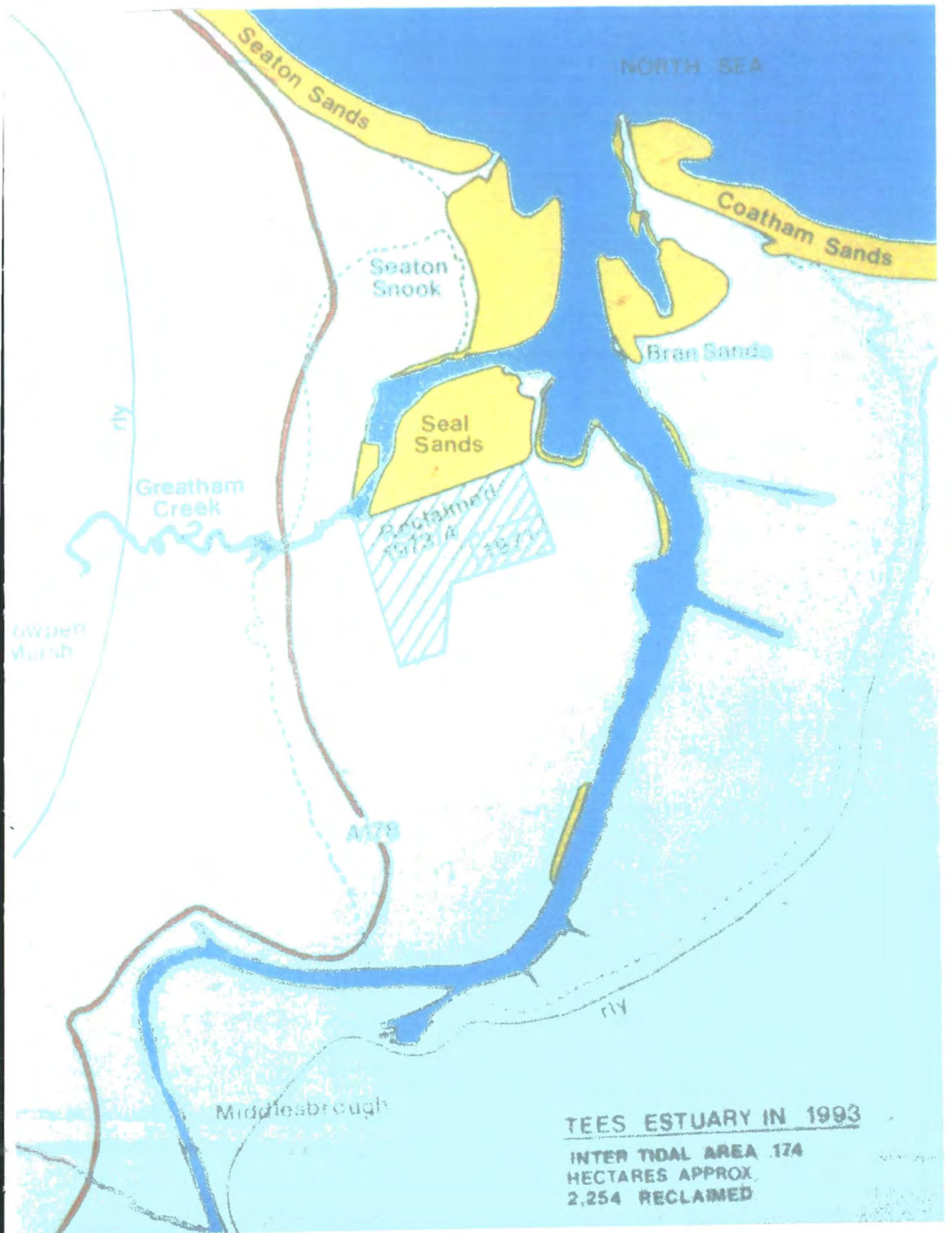
a) Original mudflats

b) Remaining mudflats

a.


Seaton Sands
Coatham Sands
Seaton Snook
Greatham Creek
Seal Sands
Middlesbrough
TEES ESTUARY IN 1861
INTER TIDAL AREA 2,428
HECTARES APPROX

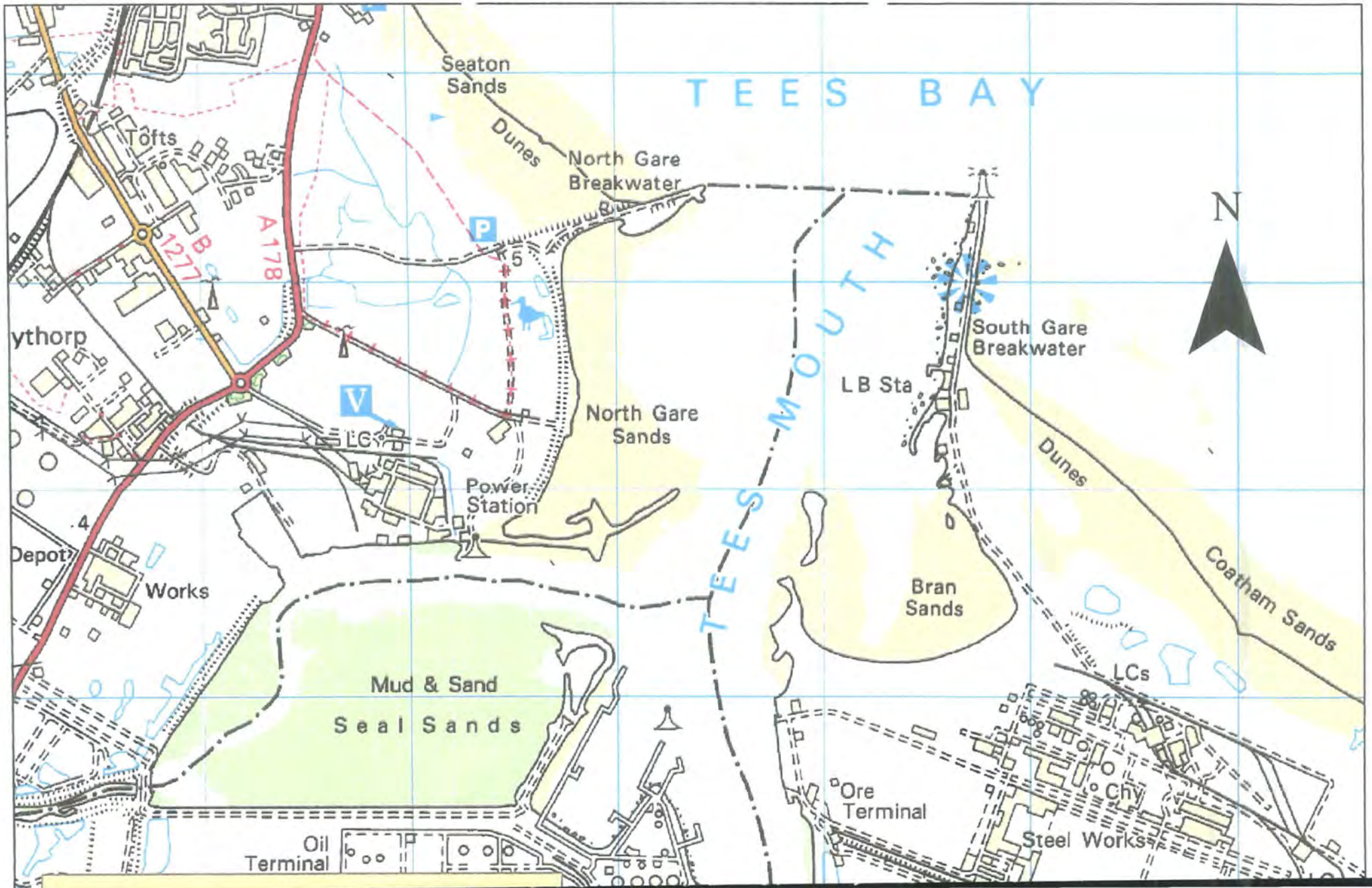
b.


Seaton Sands
NORTH SEA
Coatham Sands
Seaton Snook
Bran Sands
Seal Sands
Greatham Creek
A178
Middlesbrough
TEES ESTUARY IN 1993
INTER TIDAL AREA 174 HECTARES APPROX
2,254 RECLAIMED

Appendix B.

Map of the Tees Estuary mudflats

Mudflats on the Tees Estuary



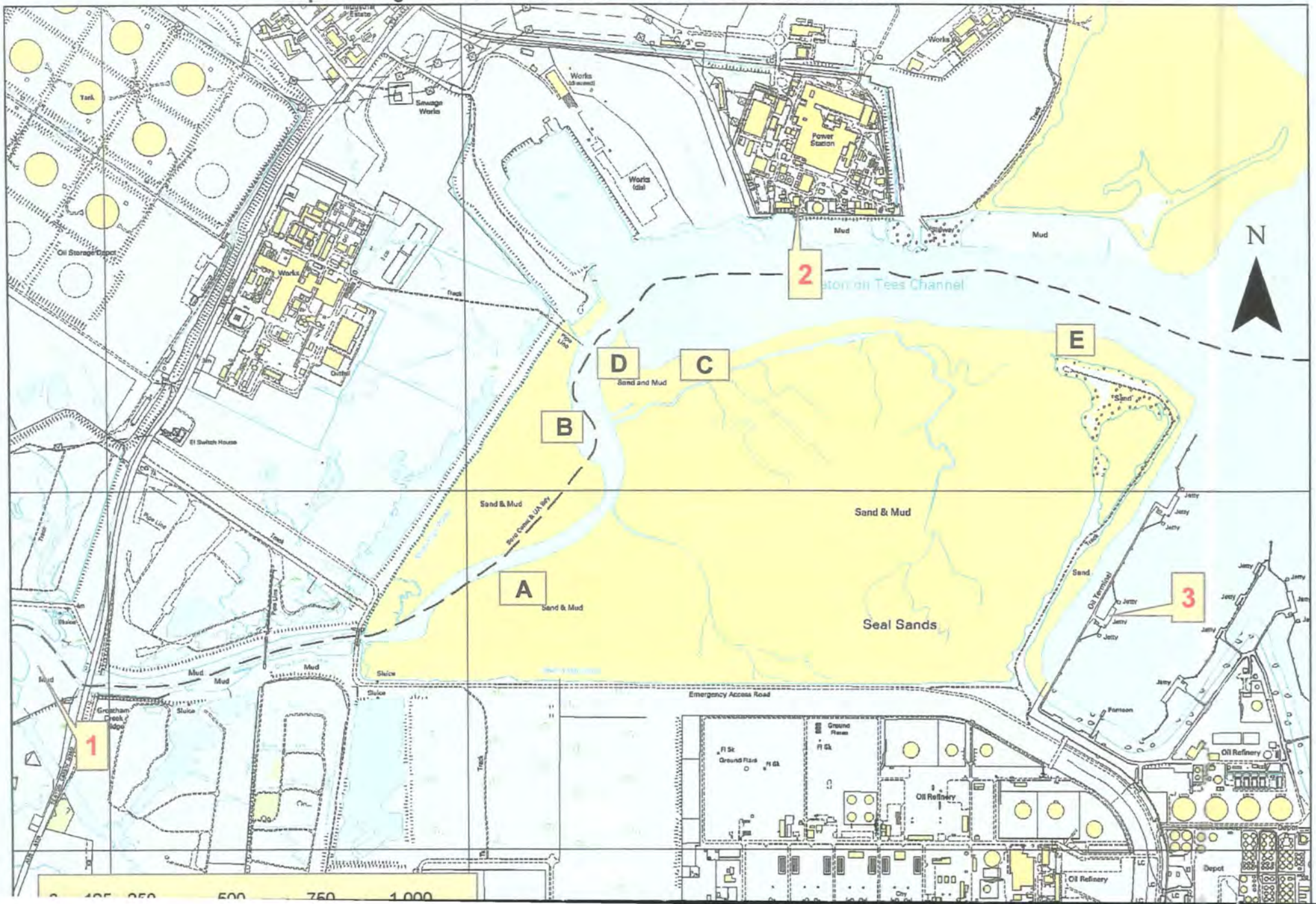
Appendix C.

Map of the field work sites used during this study (1-3) and the seal haul out sites on the Seal Sands mudflats (A-E)

1. Greatham Creek seal haul out
2. Hartlepool Power Station intake screens
3. Cormorant roost at Phillips Jetty

A-E Seal haul out, Seal Sands mudflats

Map Showing Fieldwork Sites and Seal Haul Out Sites on Seal Sands



Appendix D

i) Comparison of using otolith length and otolith width as independent variable to predict fish length for known size fish

	Otolith length			Otolith width		
Species	r^2	**df**	*p*	r^2	**df**	*p*
Cod	0.93	44	***	0.92	44	***
Flounder	0.80	80	***	0.72	79	***
Herring	0.87	35	***	0.81	37	***
Plaice	0.75	9	***	0.58	9	***
5 bearded rockling	0.92	6	***	0.62	6	*
Saithe	0.91	35	***	0.85	35	***
Lesser sandeel	0.63	39	***	0.21	39	**
Sprat	0.91	41	***	0.12	41	*
Weever	0.62	45	***	0.54	45	***
Whiting	0.94	108	***	0.89	112	***

ii) Comparison of mean fish length $^{+}/_{-}$ standard deviation for actual size fish with published linear regression equations

Species	**Actual fish length**	**Leopold *et al* (2001)**	**Härkönen (1986)**	**Tollit (1996)**	**df**
Cod	125 $^{+}/_{-}$ 50.03	130 $^{+}/_{-}$ 52.97	136 $^{+}/_{-}$ 55.60	116 $^{+}/_{-}$ 67.57	46
Flounder	167 $^{+}/_{-}$ 52.00	181 $^{+}/_{-}$ 63.12	233 $^{+}/_{-}$ 64.86	185 $^{+}/_{-}$ 66.50	82
Herring	165 $^{+}/_{-}$ 40.74	178 $^{+}/_{-}$ 39.82	231 $^{+}/_{-}$ 43.64	195 $^{+}/_{-}$ 51.88	37
Plaice	139 $^{+}/_{-}$ 40.30	154 $^{+}/_{-}$ 36.99	178 $^{+}/_{-}$ 45.15	160 $^{+}/_{-}$ 36.32	11
Saithe	148 $^{+}/_{-}$ 38.15	152 $^{+}/_{-}$ 36.99	210 $^{+}/_{-}$ 48.62	84 $^{+}/_{-}$ 32.93	37
Lesser sandeel	161 $^{+}/_{-}$ 8.17	164 $^{+}/_{-}$ 14.72	159 $^{+}/_{-}$ 23.71	180 $^{+}/_{-}$ 40.04	41
Sprat	112 $^{+}/_{-}$ 16.79	143 $^{+}/_{-}$ 18.88	123 $^{+}/_{-}$ 17.76	125 $^{+}/_{-}$ 22.64	43
Weever	119 $^{+}/_{-}$ 15.27	119 $^{+}/_{-}$ 12.02	121 $^{+}/_{-}$ 14.03	126 $^{+}/_{-}$ 13.62	47
Whiting	150 $^{+}/_{-}$ 48.07	154 $^{+}/_{-}$ 47.95	201 $^{+}/_{-}$ 50.08	171 $^{+}/_{-}$ 63.44	114

iii) Comparison of using otolith length and fish length as independent variable to predict fish mass for known size fish for both linear and power regression

		Linear equation		Power equation	
Species	**df**	**Otolith length** (r^2, p)	**Fish length** (r^2, p)	**Otolith length** (r^2, p)	**Fish length** (r^2, p)
Cod	44	0.70, ***	0.66, ***	0.93, ***	0.91, ***
Flounder	80	0.64, ***	0.64, ***	0.80, ***	0.80, ***
Herring	35	0.73, ***	0.73, ***	0.84, ***	0.81, ***
Plaice	9	0.64, **	0.64, **	0.68, **	0.53 *
5 bearded rockling	5	0.80, **	0.80, **	0.89, ***	0.86, ***
Saithe	35	0.73, ***	0.73, ***	0.91, ***	0.89, ***
Lesser sandeel	39	0.67, ***	0.67, ***	0.69, ***	0.63, ***
Sprat	41	0.85, ***	0.86, ***	0.88, ***	0.87, ***
Weever	45	0.56, ***	0.56, ***	0.62, ***	0.60, ***
Whiting	108	0.85, ***	0.85, ***	0.87, ***	0.86, ***

iv) Comparison of mean fish mass $^{+}/_{-}$ standard deviation for actual size fish with published linear regression equations

(Leopold *et al*, 2001)

Species	Actual fish mass	Leopold *et al* (2001)	df
Cod	27.4 $^{+}/_{-}$ 52.24	31.7 $^{+}/_{-}$ 44.71	46
Flounder	71.2 $^{+}/_{-}$ 81.72	76.34 $^{+}/_{-}$ 104.63	82
Herring	29.0 $^{+}/_{-}$ 19.69	29.57 $^{+}/_{-}$ 16.91	37
Plaice	32.9 $^{+}/_{-}$ 29.16	35.6 $^{+}/_{-}$ 36.76	11
5 bearded rockling	39.4 $^{+}/_{-}$ 19.55	40.7 $^{+}/_{-}$ 21.08	
Saithe	29.5 $^{+}/_{-}$ 18.59	36.72 $^{+}/_{-}$ 57.16	37
Lesser sandeel	13.8 $^{+}/_{-}$ 2.40	10.55 $^{+}/_{-}$ 3.53	41
Sprat	8.5 $^{+}/_{-}$ 5.70	13.55 $^{+}/_{-}$ 7.27	43
Weever	16.5 $^{+}/_{-}$ 6.57	19.36 $^{+}/_{-}$ 7.63	47
Whiting	31.2 $^{+}/_{-}$ 37.59	34.06 $^{+}/_{-}$ 59.18	114

Appendix E

i) Regression equations used to predict fish length from otolith length ([1]Leopold et al, 2001; [2]Härkönen, 1986)

Species	Sample size	Estimated Regression Coefficients				
		a	s.e.a	b	s.e.b	σ
Clupeiformes						
Herring [1]	285	-1.93	0.22	6.29	0.07	1.39 E + 00
Sprat	42	-9.27	6.00	58.28	2.86	
Gadiformes						
5 Bearded rockling[1]	177	-2.97	0.56	7.29	0.21	1.78 E + 00
Cod [1]	268	-6.64	0.48	3.49	0.06	2.78 E + 00
Shore Rockling						
Haddock [1]	236	-3.27	0.30	2.53	0.03	1.47 E + 00
Whiting [1]	303	0.81	0.18	1.73	0.02	1.43E + 00
Saithe [1]	85	-1.79	0.40	3.00	0.06	1.81 E + 00
Poor cod [1]	144	-3.84	0.34	2.61	0.05	8.85 E - 01
Scorpaeniformes						
Bullrout[1]	196	-1.37	0.33	3.49	0.07	1.46 E + 00
Sea scorpion[1]	38	-4.13	1.25	4.92	0.37	1.09 E + 00
Grey gurnard[1]	265	-5.09	0.37	8.55	0.13	1.93 E + 00
Perciformes						
Scad[1]	340	-0.90	0.10	3.29	0.02	1.04 E + 00
Wrasse						
Lesser weever[1]	151	-0.44	0.16	2.42	0.04	7.73 E + 01
Eelpout [1]	202	-1.98	0.48	9.24	0.23	2.09 E + 00
Butterfish [1]	94	0.89	0.35	8.71	0.25	8.66 E - 01
Lesser sandeel [1]	170	1.16	0.24	5.00	0.11	1.16 E + 00
Dragonet [1]	237	-5.48	0.35	8.41	0.14	1.45 E + 00
Pleuronectiformes						
Megrim [1]	6	0.00	-	5.84	0.19	2.01 E + 00
Long rough dab [1]	168	-1.18	0.36	4.47	0.08	1.08 E + 00
Dab[1]	261	-3.49	0.23	5.43	0.06	1.25 E + 00
Flounder [1]	324	-3.65	0.30	5.61	0.06	1.85 E + 00
Plaice [1]	405	-2.07	0.14	4.85	0.03	1.36 E + 00
[Note]Unidentified pleuronectids [2]		-25.45		53.27		
Sole [1]	344	-2.65	0.26	8.18	0.09	1.77 E + 00
Freshwater						
Perch [1]	57	-2.54	0.26	3.44	0.05	9.39 E - 01
Roach [1]	71	0.00	-	6.90	0.07	1.12 E + 00

Note: Combined data from more than one species

ii) **Regression equations used to predict fish mass from otolith length**

([1]Leopold et al, 2001; [2]Bedford et al, 1986; [3]Coull et al, 1989)

Species	Sample size	Estimated Regression Coefficients				
		a	s.e.a	b	s.e.b	σ
Clupeiformes						
Herring [1]	283	0.93	0.02	3.35	0.06	1.28 E - 02
Sprat	42	0.38		4.22		
Gadiformes						
5 Bearded rockling[1]	166	0.98	0.04	3.81	0.14	2.31 E - 02
Cod [1]	243	0.37	0.01	4.04	0.05	1.12 E - 03
Shore Rockling						
Haddock [1]	217	0.34	0.01	3.72	0.05	6.43 E - 04
Whiting [1]	297	0.37	0.00	2.95	0.03	3.71 E - 04
Saithe [1]	25	0.41	0.03	3.84	0.26	1.24 E - 03
Poor cod [1]	78	0.35	0.01	3.84	0.09	5.46 E - 04
Scorpaeniformes						
Bullrout[1]	179	0.71	0.03	3.31	0.11	6.63 E - 03
Sea scorpion[1]	33	0.67	0.03	4.22	0.26	5.55 E - 03
Grey gurnard[1]	264	0.94	0.02	4.10	0.07	2.36 E - 02
Perciformes						
Scad[1]	236	0.67	0.01	2.98	0.02	2.12 E - 03
Lesser weever[1]	124	0.49	0.01	3.31	0.09	1.41 E - 03
Eelpout [1]	202	1.16	0.03	3.84	0.11	4.48 E - 02
Butterfish [1]	94	1.33	0.03	3.39	0.09	2.40 E - 02
Lesser sandeel [1]	127	0.78	0.01	2.90	0.09	6.09 E - 03
Dragonet [1]	235	0.88	0.02	4.14	0.09	1.66 E - 02
Pleuronectiformes						
Megrim [1]	6	1.98	0.26	2.53	0.17	1.89 E - 02
Long rough dab [1]	166	0.68	0.02	3.49	0.08	4.00 E - 03
Dab[1]	227	0.69	0.01	4.01	0.06	7.25 E - 03
Flounder [1]	325	0.79	0.01	3.63	0.04	9.16 E - 03
Plaice [1]	347	0.79	0.01	3.42	0.02	5.67 E - 03
[Note]Unidentified pleuronectids [2, 3]		3.036		0.0099		
Sole [1]	222	1.13	0.02	3.63	0.04	2.42 E - 02
Freshwater						
Perch [1]	57	0.48	0.01	4.03	0.05	1.75 E - 03
Roach [1]	71	1.34	0.04	3.38	0.10	3.42 E - 02

Note: Combined data from more than one species. The independent variable is fish length.

Appendix F

Number of faecal samples collected from Greatham Creek containing prey remains, June 1999 - June 2003

Number of faeces containing otoliths	121	69.1%
Number of faeces containing other skeletal remains	10	5.7%
Number of faeces containing invertebrate remains only	7	4.0%
Total number of faeces containing prey remains	138	78.9%
Number of faeces with no prey remains	37	21.1%
Total number of faecal samples	**175**	**100%**

Appendix Gi

Number of species-specific otoliths and bones in seal faeces collected from Seal Sands, June 1999 –June 2003

Prey Type	Otoliths	Other bones	Combined	% id. from other bones
Pleuronectids	542	2	544	0.004 %
Gadids	278	2	280	0.007 %
Lesser weever	2	0	2	0 %
Lesser sandeel	6	0	6	0 %
Dragonet	11	1	12	0.08 %
Clupeids	44	21	65	32.3 %

Appendix Gii

Number of species-specific otoliths and bones in cormorant pellets collected from Seal Sands, January 2000-December 2002

Prey Type	Otoliths	Other bones	Combined	% id. from other bones
Pleuronectids	1105	0	1105	0%
Gadids	851	0	851	0%
Lesser weever	704	1	705	0.1%
Lesser sandeel	194	1	195	0.5%
Cyprinids	124	14	138	10.1%
Dragonet	87	9	96	9.4%
Perch	58	0	58	0%
Clupeids	18	6	24	25%
Bullrout	21	1	22	4.5%
Scad	18	0	18	0%
Wrasse	9	0	9	0%
Eelpout	5	2	7	28.6%
Butterfish	1	1	2	50%
Gurnards	2	0	2	0%
Gobiids	1	0	1	0%
Megrim	1	0	1	0%
Sole	1	0	1	0%

Appendix H

Comparison of the main prey family groups consumed by i) seals and ii) cormorants calculated by numerical importance of prey, the modified frequency of occurrence and the percentage biomass (percentage biomass could not be calculated for Crangon and crabs)

i)

Species	Numerical Frequency	MFO (%)	% Biomass
Clupeidae	6.4	8.19	3.04
Gadidae	29.2	22.03	79.67
Weever	0.2	0.57	0.05
Eelpout	0.3	0.85	0.17
Sandeel	0.6	0.57	0.22
Dragonet	1.2	1.41	2.43
Pleuronectidae	56.5	14.97	14.41
Crangon	1.2	2.17	/
Crab	4.3	4.6	/

ii)

Species	Numerical Frequency	MFO (%)	% Biomass
Clupeidae	0.43	2.14	1.25
Gadidae	21.65	19.01	47.57
Cottidae	0.53	1.65	2.21
Scad	0.43	1.07	2.35
Weever	17.91	7.49	4.96
Eelpout	0.23	0.41	0.04
Sandeel	4.94	2.80	1.94
Dragonet	2.19	4.61	2.23
Pleuronectidae	28.12	16.96	27.91
Cyprinids	3.13	2.88	6.52
Perch	1.45	1.40	2.62
Crangon	3.65	4.86	/
Crab	0.81	2.06	/

Appendix I

i) Bi-monthly counts of whole body samples collected for each of common shrimp, whiting, flounder and sprat, 2000-2003

	Jan - Feb	Mar-Apr	May-Jun	Jul-Aug	Sept-Oct	Nov-Dec	Total
Common Shrimp	26	30	23	47	39	17	182
Whiting	36	40	24	47	61	54	262
Flounder	31	59	42	27	46	43	248
Sprat	26	39	37	45	55	45	247

ii) Counts of winter and summer samples of cod, plaice, herring and shore crab, 2000-2003

	Summer (Mar-Aug)	Winter (Sept-Dec)	Total
Shore crab	51	55	106
Cod	7	33	40
Plaice	16	20	36
Herring	60	96	156

iii) Counts of winter and summer samples of whiting and flounder for Hg analysis, 2000-2003

Species	Year	Summer	Winter	Total
Whiting	2000	0	38	**38**
	2001	7	3	**10**
	2002	20	33	**53**
	Total	**27**	**74**	**101**
Flounder	2000	2	2	**4**
	2001	6	5	**11**
	2002	12	17	**29**
	Total	**20**	**24**	**44**

Appendix J

Comparison of metal concentrations between pairs of six fish species from the Tees Estuary (Mann-Whitney U test)

i. Zn concentrations

	Cod	Flounder	Plaice	Sprat	Herring
Whiting	NS	*** Fl	*** Pl	*** Sp	***Herr
Cod	***Fl	***Pl	***Sp	***Herr	
Flounder	NS	***Fl	***Fl		
Plaice	NS	NS			
Sprat	NS				

ii. Cu concentrations

	Cod	Flounder	Plaice	Sprat	Herring
Whiting	NS	***Fl	NS	***Sp	***Herr
Cod	***Fl	NS	*Sp	**Herr	
Flounder	NS	NS	NS		
Plaice	NS	NS			
Sprat	NS				

iii. Pb concentrations

	Cod	Flounder	Plaice	Sprat	Herring
Whiting	***Cod	***Fl	***Pl	**Sp	***Herr
Cod	NS	NS	NS	NS	
Flounder	NS	*** Fl	*** Fl		
Plaice	** Pl	* Pl			
Sprat	NS				

iv. Cd concentrations

	Cod	Flounder	Plaice	Sprat	Herring
Whiting	NS	***Fl	NS	**Sp	***Herr
Cod	***Fl	* Pl	NS	***Herr	
Flounder	NS	NS	NS		
Plaice	NS	NS			
Sprat	NS				

v. As concentrations

	Cod	Flounder	Plaice	Sprat	Herring
Whiting	NS	NS	NS	NS	NS
Cod	NS	* Pl	NS	NS	
Flounder	* Pl	* Sp	NS		
Plaice	NS	NS			
Sprat	NS				

vi. Cr concentrations

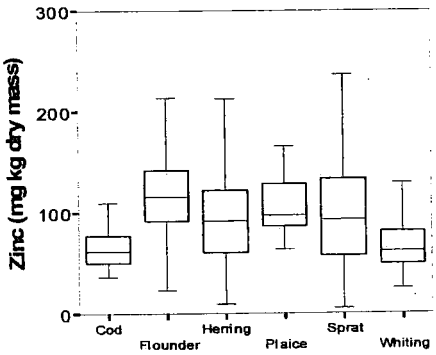
	Cod	Flounder	Plaice	Sprat	Herring
Whiting	*Cod	** Fl	***Pl	NS	NS
Cod	NS	** Pl	**Cod	*Cod	
Flounder	** Pl	***Fl	* Fl		
Plaice	*** Pl	*** Pl			
Sprat	NS				

Appendix K

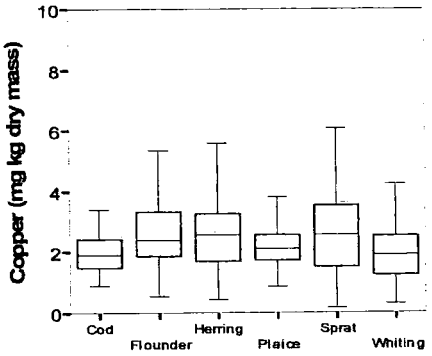
Comparison of metal concentrations between different fish species

i) Zn ii) Cu iii) Pb iv) Cd v) As vi) Cr

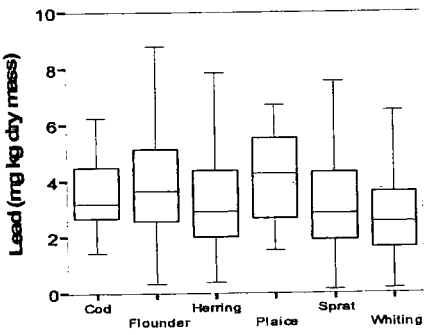
i)



ii)



iii)



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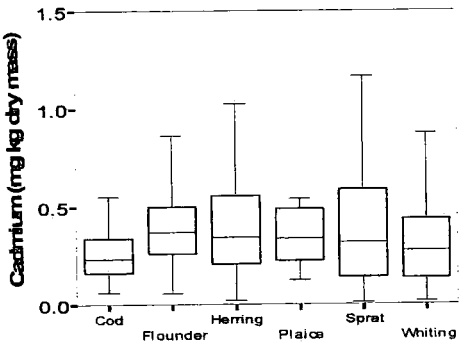

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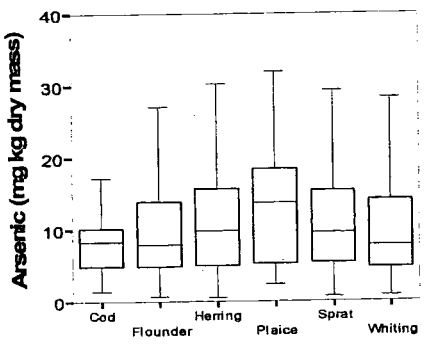

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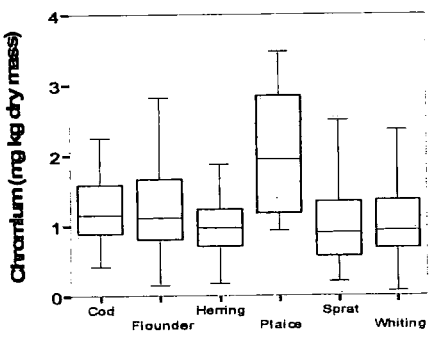
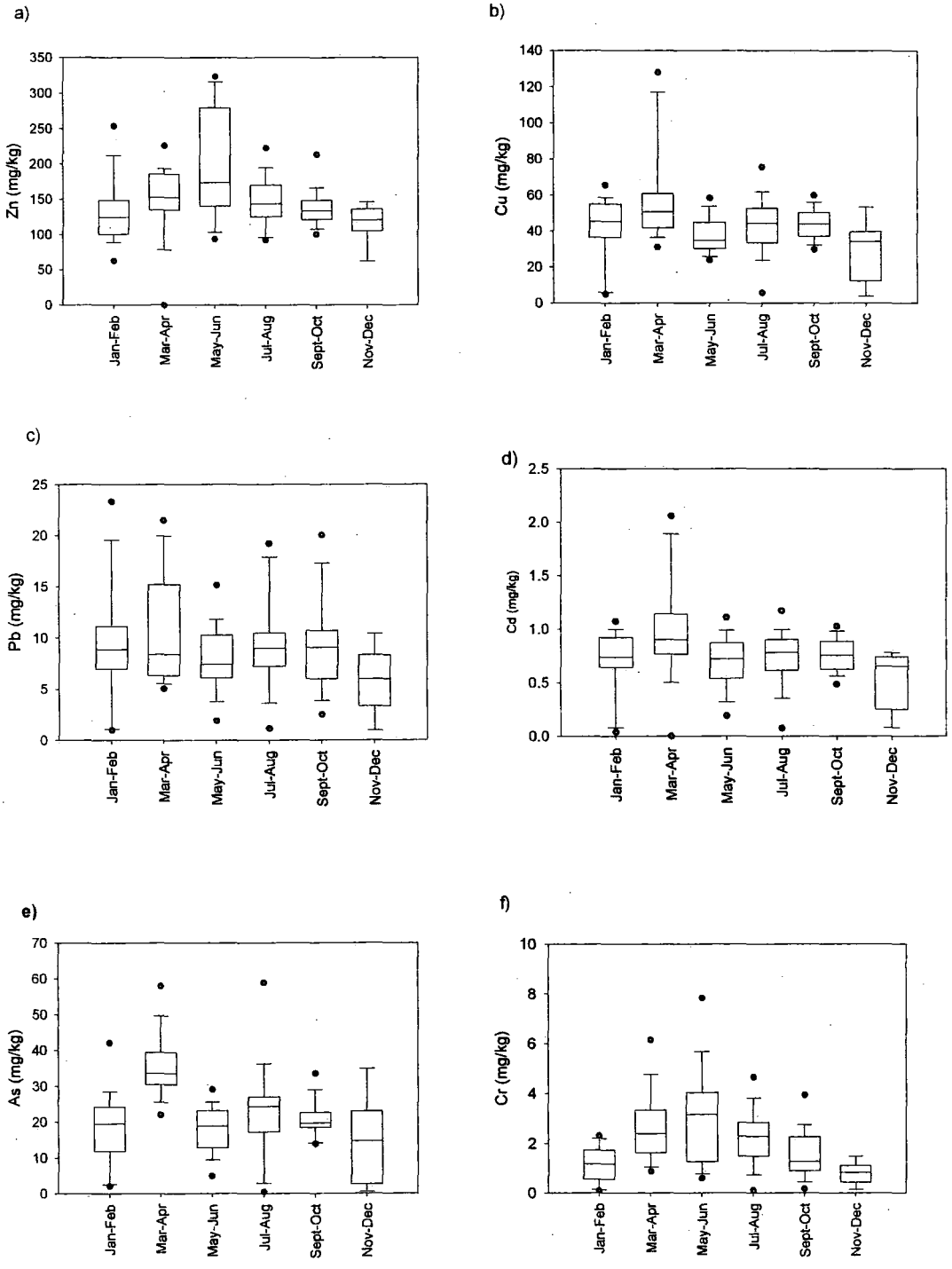


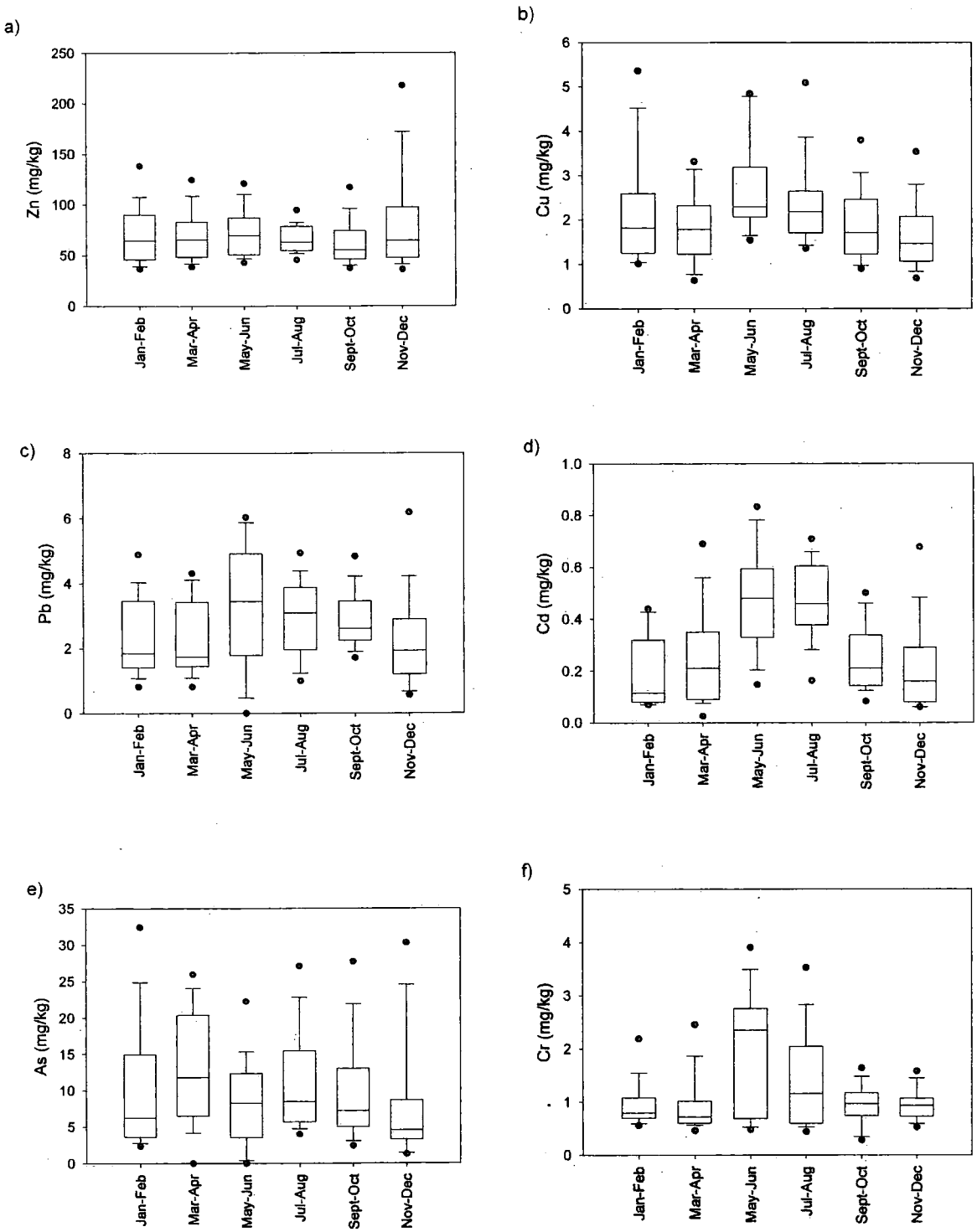
Appendix L.

i) Comparison of seasonal metal concentrations in common shrimp
a) Zn b) Cu c) Pb d) Cd e) As f) Cr



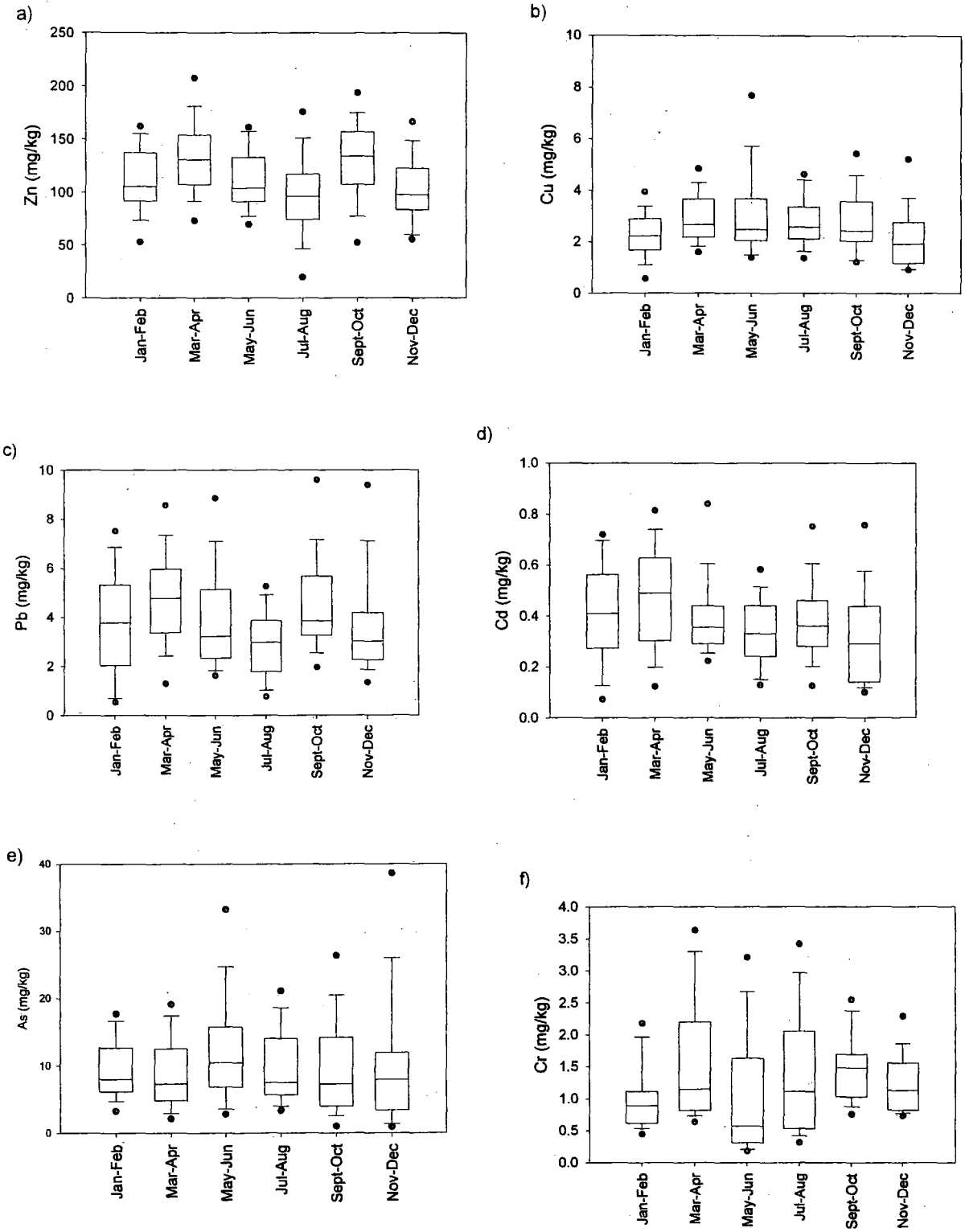
ii) Comparison of seasonal metal concentrations in whiting

a) Zn b) Cu c) Pb d) Cd e) As f) Cr

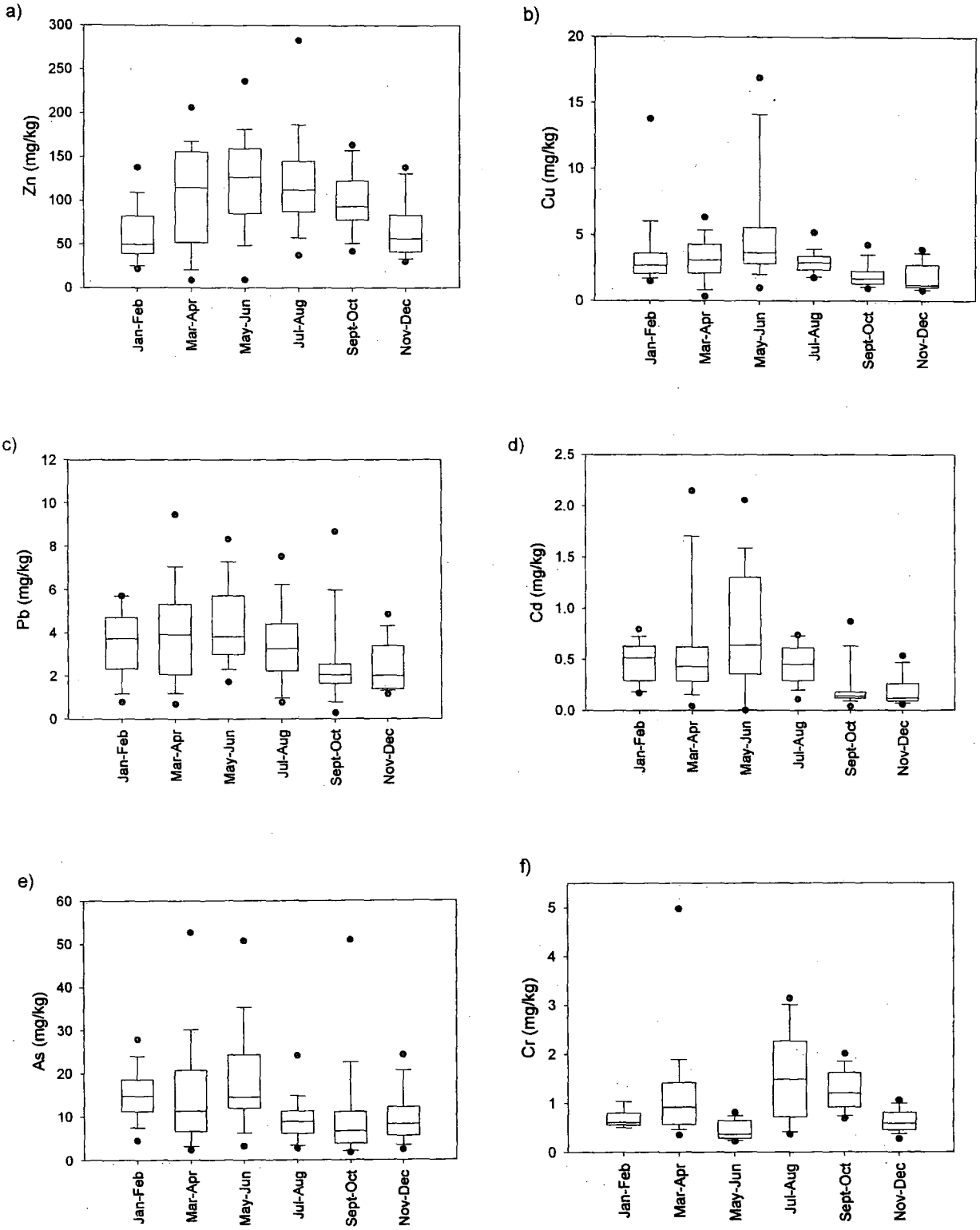


iii) Comparison of seasonal metal concentrations in flounder

a) Zn b) Cu c) Pb d) Cd e) As f) Cr

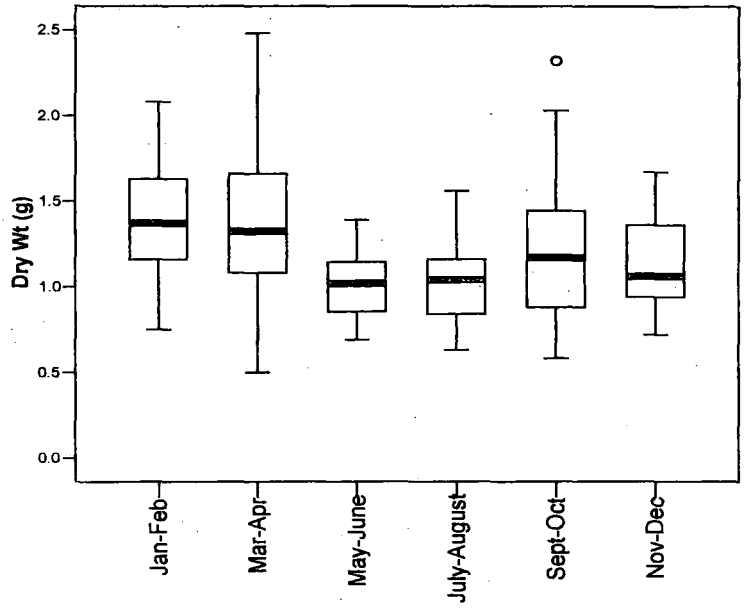


iv) Comparison of seasonal metal concentrations in sprat
a) Zn b) Cu c) Pb d) Cd e) As f) Cr

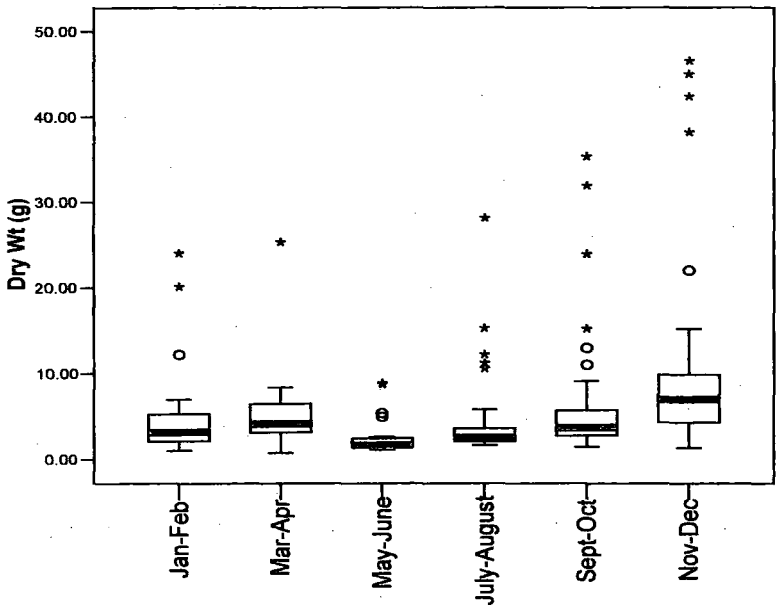


Appendix M

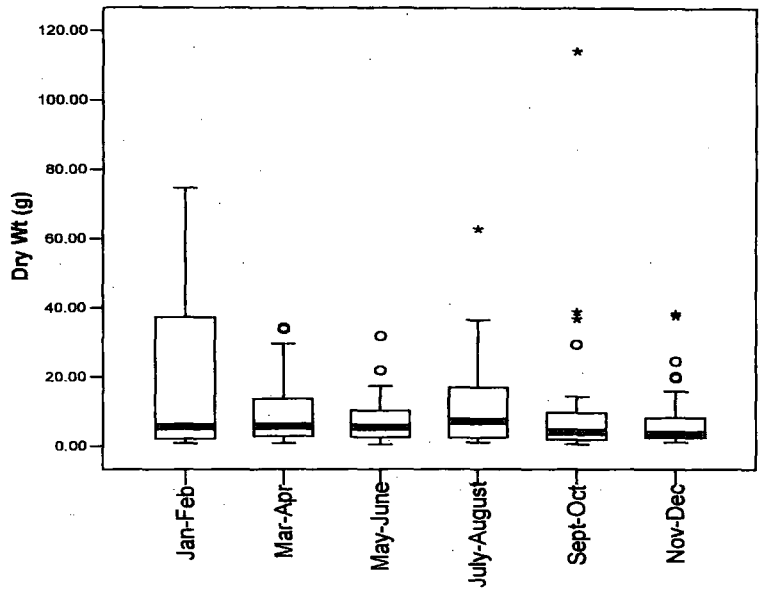
i) Variation in seasonal body size of common shrimp (dry mass (g))



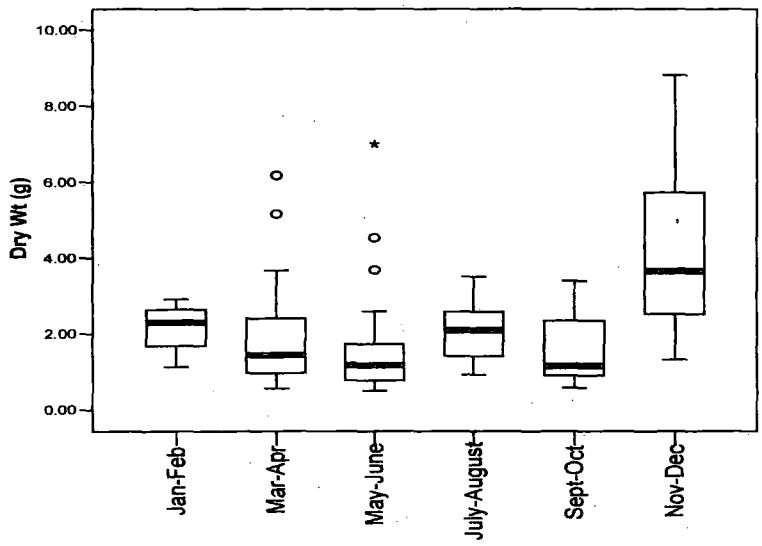
ii) Variation in seasonal body size of whiting (dry mass (g))



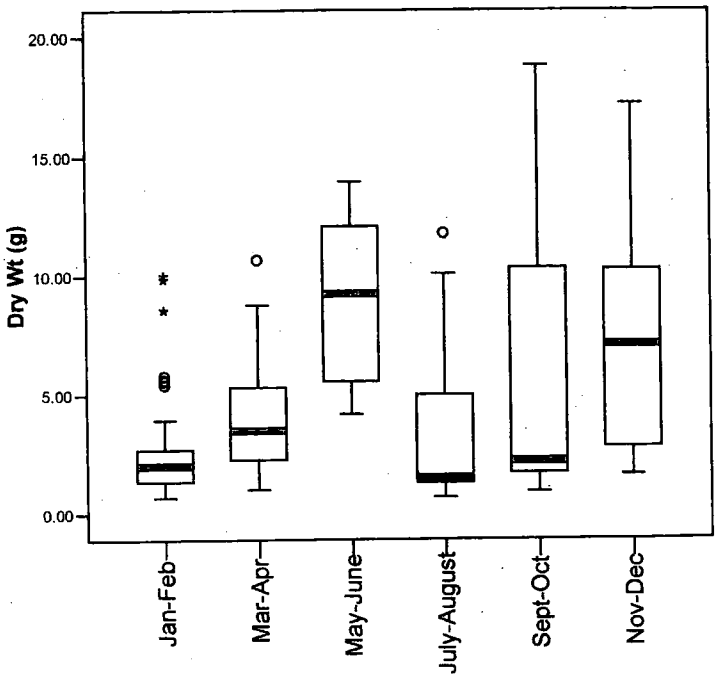
iii) Variation in seasonal body size of flounder (dry mass (g))



iv) Variation in seasonal body size of sprat (dry mass (g))

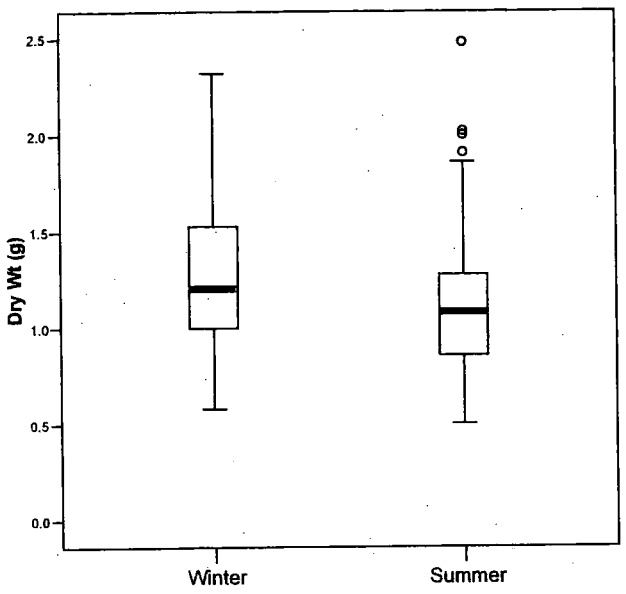


v) Variation in seasonal body size of herring (dry mass (g))

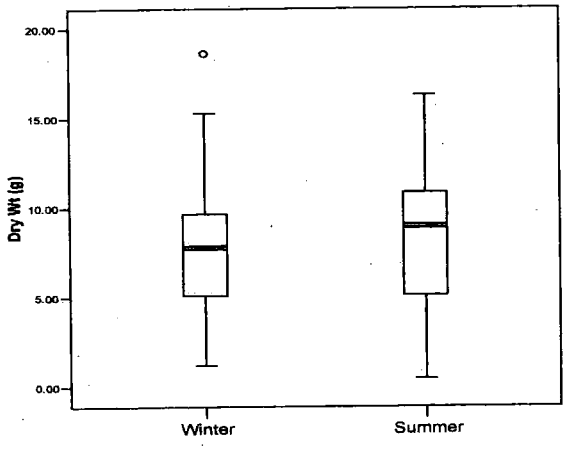


Appendix N

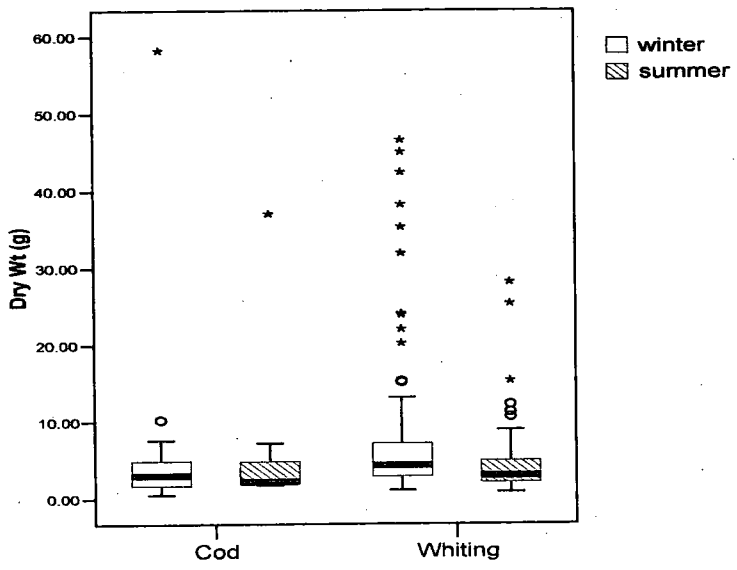
i) Variation in common shrimp dry mass (g) between winter and summer



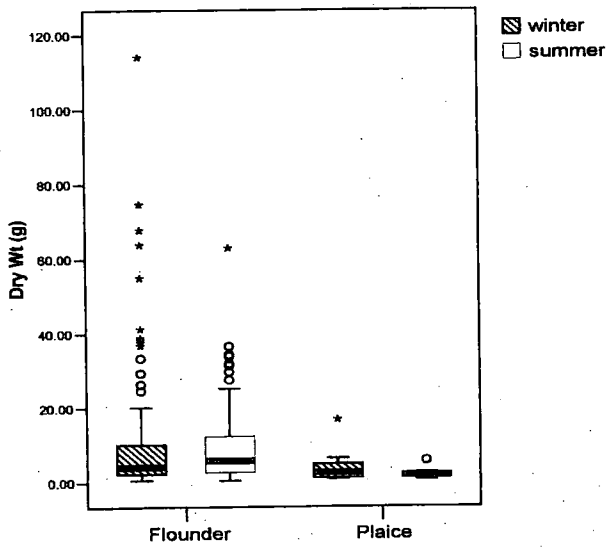
ii) Variation in shore crab dry mass (g) between winter and summer



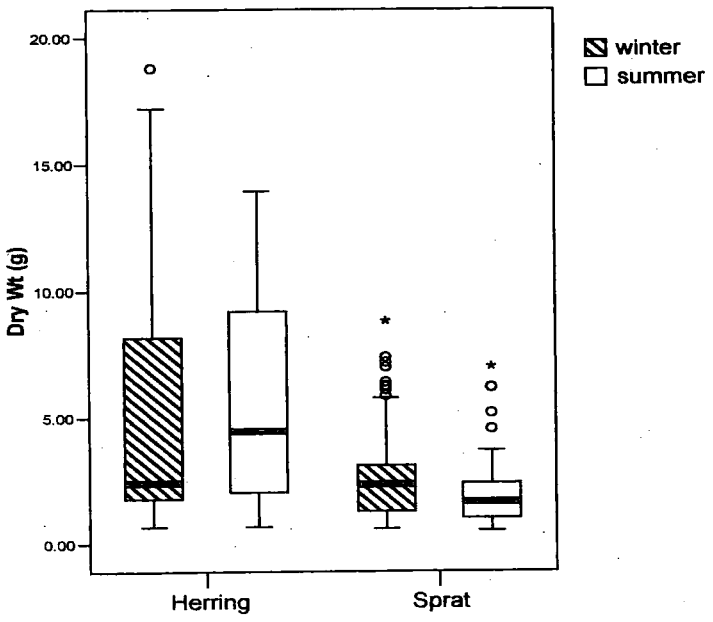
iii) Variation in whiting and cod dry mass (g) between winter and summer



iv) Variation in flounder and plaice dry mass (g) between winter and summer



v) Variation in sprat and herring dry mass (g) between winter and summer



Appendix O

i) Seasonal correlation between body size and metal concentrations in common shrimp

a. January-February

	Zn N=26	Cu N=26	Pb N=25	Cd N=26	As N=26	Cr N=25
Dry Mass	NS	NS	NS	NS	0.389 *	NS
Length	NS	NS	NS	NS	NS	NS
Wet Mass	NS	NS	NS	NS	NS	NS

b. March-April

	Zn N=26	Cu N=28	Pb N=28	Cd N=26	As N=28	Cr N=28
Dry Mass	-0.440 *	NS	NS	-0.401 *	NS	-0.737 ***
Length	-0.423 *	NS	NS	NS	NS	-0.618 ***
Wet Mass	NS	NS	NS	-0.395 *	NS	-0.620 ***

c. May-June

	Zn N=23	Cu N=23	Pb N=23	Cd N=23	As N=23	Cr N=23
Dry Mass	-0.619 **	NS	NS	-0.507 *	NS	-0.441 *
Length	-0.715 ***	NS	-0.498 *	-0.566 **	NS	NS
Wet Mass	-0.562 **	NS	NS	NS	NS	NS

d. July-August

	Zn N=27	Cu N=47	Pb N=47	Cd N=47	As N=26	Cr N=31
Dry Mass	NS	NS	NS	NS	NS	NS
Length	NS	-0.430 **	-0.324 *	NS	NS	NS
Wet Mass	NS	NS	NS	NS	NS	-0.410 *

e. September-October

	Zn N=38	Cu N=38	Pb N=38	Cd N=38	As N=38	Cr N=37
Dry Mass	NS	NS	-0.395 *	NS	NS	NS
Length	NS	NS	NS	NS	NS	-0.485 **
Wet Mass	NS	NS	NS	-0.459 **	NS	-0.416 **

f. November-December

	Zn N=17	Cu N=17	Pb N=17	Cd N=17	As N=16	Cr N=17
Dry Mass	NS	NS	NS	NS	NS	NS
Length	NS	NS	NS	NS	NS	NS
Wet Mass	NS	NS	NS	NS	NS	NS

ii) Seasonal correlation between body size and metal concentrations in whiting

a. January-February

	Zn N=36	Cu N=36	Pb N=34	Cd N=36	As N=35	Cr N=26
Dry Mass	-0.356 *	-0.758 ***	-0.730 ***	-0.733 ***	-0.761 ***	NS
Length	NS	-0.565 ***	-0.422 *	-0.491 **	-0.583 ***	-0.640 ***
Wet Mass	NS	-0.265***	-0.225***	-0.217***	NS	-0.359***

b. March-April

	Zn N=40	Cu N=40	Pb N=40	Cd N=38	As N=37	Cr N=27
Dry Mass	NS	NS	NS	NS	NS	NS
Length	NS	NS	NS	NS	NS	NS
Wet Mass	NS	NS	NS	-0.409 *	NS	NS

c. May-June

	Zn N=24	Cu N=24	Pb N=22	Cd N=24	As N=16	Cr N=14
Dry Mass	NS	NS	NS	-0.409 *	NS	NS
Length	NS	NS	NS	NS	NS	-0.633 *
Wet Mass	NS	-0.423*	NS	-0.461 *	NS	NS

d. July-August

	Zn N=47	Cu N=47	Pb N=46	Cd N=47	As N=33	Cr N=39
Dry Mass	NS	NS	NS	0.407 **	-0.383 *	-0.357 *
Length	NS	NS	NS	0.316 *	NS	-0.341 *
Wet Mass	NS	NS	NS	-0.409 *	NS	-0.359 *

e. September-October

	Zn N=61	Cu N=61	Pb N=61	Cd N=59	As N=60	Cr N=40
Dry Mass	NS	NS	NS	NS	NS	-0.475 **
Length	NS	NS	NS	NS	NS	-0.481 **
Wet Mass	NS	NS	NS	NS	NS	-0.518 ***

f. November-December

	Zn N=54	Cu N=54	Pb N=54	Cd N=54	As N=45	Cr N=45
Dry Mass	0.323 *	NS	NS	NS	NS	NS
Length	NS	NS	NS	NS	0.310 *	NS
Wet Mass	0.277 *	NS	NS	-0.461 *	0.319 *	NS

iii) Seasonal correlation between body size and metal concentrations in flounder

a. **January-February**

	Zn N=30	Cu N=30	Pb N=31	Cd N=31	As N=24	Cr N=23
Dry Mass	NS	NS	NS	-0.391 *	NS	NS
Length	-0.386 *	NS	NS	-0.377 *	NS	NS
Wet Mass	NS	NS	NS	-0.357 *	NS	NS

b. **March-April**

	Zn N=58	Cu N=59	Pb N=59	Cd N=59	As N=56	Cr N=45
Dry Mass	-0.468 ***	-0.446 ***	NS	NS	NS	NS
Length	-0.442 ***	-0.421 ***	NS	NS	NS	NS
Wet Mass	-0.454 ***	-0.441 ***	NS	NS	NS	NS

c. **May-June**

	Zn N=42	Cu N=42	Pb N=42	Cd N=42	As N=41	Cr N=18
Dry Mass	-0.325 *	NS	-0.325 *	-0.414 **	NS	NS
Length	NS	NS	NS	-0.376 *	NS	NS
Wet Mass	-0.322*	-0.423*	NS	-0.414 **	NS	NS

d. **July-August**

	Zn N=26	Cu N=26	Pb N=27	Cd N=27	As N=25	Cr N=13
Dry Mass	-0.466 *	NS	NS	NS	NS	NS
Length	-0.474 *	NS	NS	NS	NS	NS
Wet Mass	-0.522**	NS	NS	NS	NS	NS

e. **September-October**

	Zn N=46	Cu N=46	Pb N=46	Cd N=46	As N=39	Cr N=27
Dry Mass	NS	NS	-0.309 *	NS	NS	-0.627 ***
Length	NS	NS	NS	NS	NS	-0.642 ***
Wet Mass	NS	NS	NS	NS	NS	-0.627 ***

f. **November-December**

	Zn N=43	Cu N=43	Pb N=42	Cd N=43	As N=43	Cr N=35
Dry Mass	NS	NS	NS	NS	NS	NS
Length	NS	NS	NS	NS	NS	NS
Wet Mass	NS	NS	NS	NS	NS	NS

iv) Seasonal correlation between body size and metal concentrations in sprat

a. January-February

	Zn N=26	Cu N=26	Pb N=25	Cd N=26	As N=21	Cr N=7
Dry Mass	NS	NS	-0.431 *	NS	-0.472 *	NS
Length	NS	NS	NS	NS	-0.446 *	NS
Wet Mass	NS	NS	-0.430 *	NS	-0.583 ***	NS

b. March-April

	Zn N=38	Cu N=38	Pb N=39	Cd N=37	As N=24	Cr N=15
Dry Mass	-0.375 *	-0.739 ***	-0.335 *	-0.476 **	-0.618 ***	-0.658 **
Length	NS	-0.544 ***	-0.402 *	-0.376 *	-0.600 **	-0.554 *
Wet Mass	NS	-0.471 **	-0.339 *	-0.392 *	-0.608 **	-0.629 *

c. May-June

	Zn N=37	Cu N=37	Pb N=36	Cd N=33	As N=24	Cr N=15
Dry Mass	-0.328 *	NS	NS	NS	NS	NS
Length	0.411 *	NS	NS	NS	NS	NS
Wet Mass	NS	NS	NS	-0.455 **	NS	NS

d. July-August

	Zn N=45	Cu N=45	Pb N=43	Cd N=44	As N=26	Cr N=28
Dry Mass	-0.605 ***	-0.528 ***	-0.615 ***	-0.634 ***	NS	NS
Length	-0.631 ***	NS	-0.479 ***	-0.504 ***	NS	-0.556 **
Wet Mass	-0.492 ***	-0.304 *	-0.483 ***	-0.395 **	NS	NS

e. September-October

	Zn N=53	Cu N=55	Pb N=53	Cd N=54	As N=54	Cr N=40
Dry Mass	-0.455 ***	-0.571 ***	NS	NS	NS	-0.529 ***
Length	-0.278 *	-0.496 ***	NS	NS	NS	NS
Wet Mass	-0.315 *	-0.483 ***	NS	NS	NS	-0.389 *

f. November-December

	Zn N=45	Cu N=45	Pb N=45	Cd N=45	As N=43	Cr N=37
Dry Mass	-0.603 ***	-0.587 ***	-0.561 ***	-0.632 ***	NS	-0.487 **
Length	-0.344 *	NS	-0.342 *	-0.300 *	NS	NS
Wet Mass	-0.495 ***	-0.391 **	-0.470 ***	-0.444 **	NS	-0.443 **

v) Seasonal correlation between body size and metal concentrations in herring

a. **January-February**

	Zn N=50	Cu N=50	Pb N=48	Cd N=49	As N=37	Cr N=7
Dry Mass	-0.509 ***	NS	NS	NS	NS	NS
Length	-0.381 **	NS	NS	NS	NS	NS
Wet Mass	-0.479 ***	NS	NS	NS	NS	NS

b. **March-April**

	Zn N=21	Cu N=22	Pb N=21	Cd N=22	As N=19	Cr N=16
Dry Mass	NS	NS	NS	NS	-0.589 **	NS
Length	NS	NS	NS	NS	-0.546 *	NS
Wet Mass	NS	NS	NS	NS	-0.589 **	NS

c. **May-June**

	Zn N=21	Cu N=21	Pb N=19	Cd N=21	As N=21	Cr N=18
Dry Mass	NS	-0.451 *	NS	NS	NS	NS
Length	NS	NS	NS	NS	NS	NS
Wet Mass	NS	NS	NS	NS	NS	NS

d. **July-August**

	Zn N=17	Cu N=17	Pb N=16	Cd N=17	As N=11	Cr N=16
Dry Mass	-0.747 ***	-0.543 *	-0.826 ***	-0.671 **	NS	-0.787 ***
Length	-0.736 ***	-0.552 *	-0.810 ***	-0.620 **	NS	-0.731 ***
Wet Mass	-0.816 ***	-0.605 **	-0.803 ***	-0.578 *	NS	-0.679 **

e. **September-October**

	Zn N=10	Cu N=11	Pb N=11	Cd N=11	As N=11	Cr N=7
Dry Mass	NS	NS	-0.745 **	NS	NS	NS
Length	-0.636 *	NS	-0.636 *	NS	NS	NS
Wet Mass	NS	NS	-0.655 *	NS	NS	NS

f. **November-December**

	Zn N=35	Cu N=35	Pb N=35	Cd N=33	As N=32	Cr N=21
Dry Mass	-0.628 ***	NS	NS	-0.361 *	NS	-0.485 *
Length	-0.594 ***	0.439 **	NS	-0.445 **	NS	NS
Wet Mass	-0.572 ***	-0.335 *	NS	-0.371 *	NS	NS

Appendix P

i) Effect of body size on winter and summer metal concentrations in common shrimp from the Tees Estuary

a. summer

	Zn N=89	Cu N=111	Pb N=111	Cd N=109	As N=90	Cr N=95
Dry Mass	-0.520 ***	NS	NS	NS	NS	NS
Length	-0.559***	-0.234 *	NS	NS	NS	NS
Wet Mass	-0.515***	-0.222 **	NS	NS	NS	NS

b. winter

	Zn N=86	Cu N=86	Pb N=85	Cd N=86	As N=84	Cr N=84
Dry Mass	NS	NS	NS	0.288**	0.225 *	NS
Length	NS	NS	NS	NS	NS	0.282 **
Wet mass	NS	NS	NS	NS	NS	0.266 *

ii) Effect of body size on winter and summer metal concentrations in shore crab from the Tees Estuary

a. summer

	Zn N=58	Cu N=58	Pb N=59	Cd N=59	As N=46	Cr N=59
Dry Mass	NS	-0.266 *	NS	NS	0.389 *	NS
Length	NS	NS	-0.333 **	NS	NS	-0.272 *
Wet Mass	NS	-0.318 *	-0.433 ***	NS	NS	-0.396 **

b. winter

	Zn N=70	Cu N=70	Pb N=70	Cd N=70	As N=68	Cr N=69
Dry Mass	NS	NS	NS	NS	NS	NS
Length	NS	NS	-0.345 **	-0.402 ***	NS	-0.411 ***
Wet Mass	-0.272 *	NS	-0.316 **	-0.421 ***	NS	-0.397 ***

iii) Effect of body size on winter and summer metal concentrations in whiting from the Tees Estuary

a. Summer

	Zn N=111	Cu N=111	Pb N=108	Cd N=109	As N=91	Cr N=80
Dry Mass	NS	-0.413***	-0.195*	NS	NS	-0.385***
Length	NS	-0.320 ***	-0.224*	NS	NS	-0.410***
Wet Mass	NS	-0.312 ***	-0.207*	NS	NS	-0.370***

b. Winter

	Zn N=151	Cu N=151	Pb N=149	Cd N=149	As N=140	Cr N=111
Dry Mass	NS	-0.200*	-0.250**	-0.196*	-0.196 *	-0.293**
Length	NS	NS	-0.205*	NS	NS	0.323 ***
Wet Mass	NS	NS	-0.211**	NS	NS	0.330 ***

iv) Effect of body size on winter and summer metal concentrations in flounder from the Tees Estuary

a. summer

	Zn N=126	Cu N=127	Pb N=128	Cd N=128	As N=122	Cr N=76
Dry Mass	-0.372***	-0.260 **	NS	NS	NS	NS
Length	-0.314 ***	-0.227 **	NS	NS	NS	NS
Wet Mass	-0.381 ***	-0.238 **	NS	NS	NS	NS

b. winter

	Zn N=119	Cu N=119	Pb N=119	Cd N=120	As N=106	Cr N=85
Dry Mass	NS	NS	NS	NS	NS	NS
Length	NS	NS	NS	NS	NS	0.266 *
Wet Mass	NS	NS	NS	NS	NS	0.242 *

v) Effect of body size on winter and summer metal concentrations in sprat from the Tees Estuary

a. summer

	Zn N=126	Cu N=127	Pb N=128	Cd N=128	As N=122	Cr N=76
Dry Mass	-0.635 **	NS	NS	NS	NS	NS
Length	-0.599 **	NS	-0.631**	-0.627**	NS	NS
Wet Mass	-0.629 **	NS	-0.626**	-0.607**	NS	NS

b. winter

	Zn N=19	Cu N=20	Pb N=20	Cd N=20	As N=20	Cr N=9
Dry Mass	-0.491*	NS	NS	NS	NS	-0.817**
Length	-0.462*	NS	NS	NS	NS	-0.750 *
Wet Mass	NS	NS	NS	NS	NS	-0.717 *

vi) Effect of body size on winter and summer metal concentrations in herring from the Tees Estuary

a. summer

	Zn N=120	Cu N=120	Pb N=118	Cd N=114	As N=74	Cr N=53
Dry Mass	-0.433 ***	-0.561***	-0.421***	-0.464***	-0.402***	NS
Length	NS	-0.357***	-0.433 ***	-0.426 ***	-0.418***	NS
Wet Mass	-0.629 **	-0.367***	-0.391 ***	-0.443***	-0.427***	NS

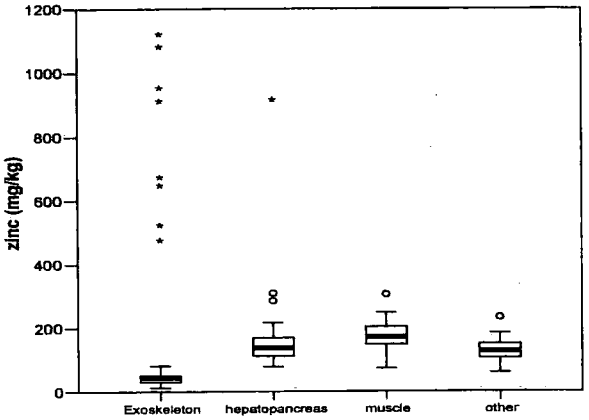
b. winter

	Zn N=124	Cu N=126	Pb N=123	Cd N=125	As N=118	Cr N=84
Dry Mass	-0.533***	-0.463***	-0.257 **	-0.299 ***	NS	-0.724 ***
Length	-0.430 ***	-0.267 **	NS	NS	NS	-0.569 ***
Wet Mass	-0.526 ***	-0.300 ***	NS	NS	NS	-0.716 ***

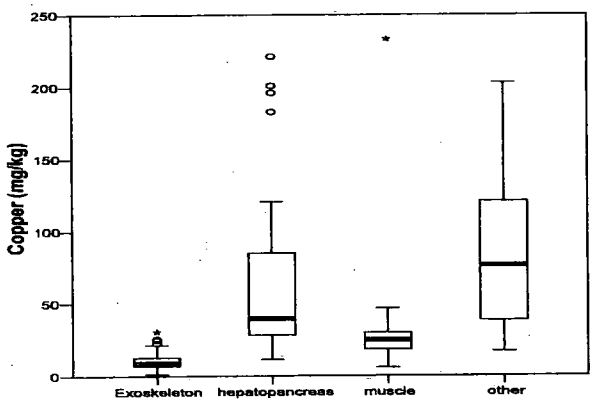
Appendix Q

Boxplots of median metal concentrations in different tissues in shore crab. i) Zn ii) Cu iii) Pb iv) Cd v) As vi) Cr

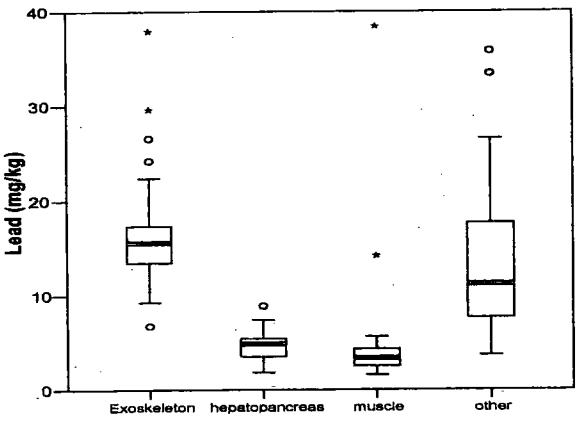
i)



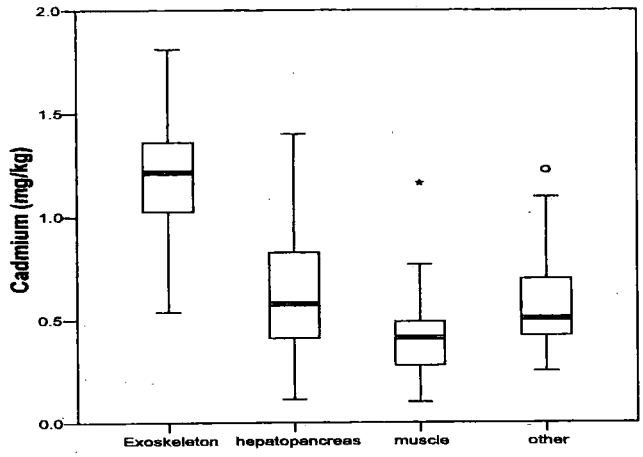
ii)



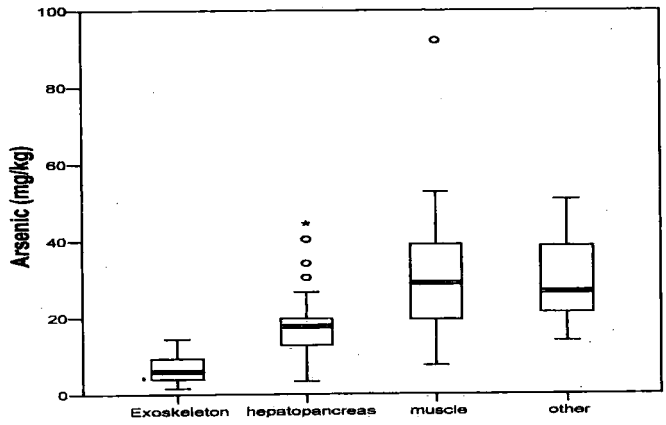
iii)



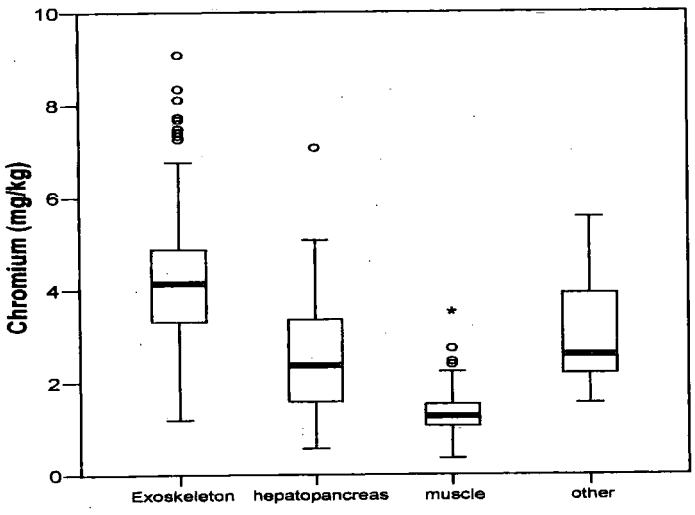
iv)


Cadmium (mg/kg)
2.0
1.5
1.0
0.5
0.0
Exoskeleton
hepatopancreas
muscle
other

v)


Arsenic (mg/kg)
100
80
60
40
20
0
Exoskeleton
hepatopancreas
muscle
other

vi)


Chromium (mg/kg)
10
8
6
4
2
0
Exoskeleton
hepatopancreas
muscle
other